EFFECT OF DELAYED LEUKOREDUCTION ON RBC AFTER PROLONG STORAGE BY USE OF INLINE FILTER

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Dedicated

to

my family

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Dr. Harleen Kaur

LIST OF ABBREVIATIONS

WBC:	White blood cells
HLA:	Human leukocyte antigen
RBC:	Red blood Cells
RBCC:	Red blood cell concentrates
DPG:	diphosphoglycerate
ATP:	Adenosine tri phosphate
CMV:	Cytomegalovirus
Na:	Sodium
K:	Potassium
GVHD:	Graft versus Host Disease
TA- GVHD	Transfusion associated graft versus host disease
FNHTR:	Febrile non- haemolytic transfusion reactions.
IL:	Interleukins
ACD:	Adenine, citrate and dextrose
CPD:	Citrate phosphate and dextrose
SAG-M	Saline, adenine, glucose and mannitol
ROS	Reactive Oxygen Species
HPLC	High performance liquid chromatography
TMB	Tetramethylbenzidine
ECF:	Extracellular fluid
ICF:	Intracellular fluid
s/s:	signs and symptoms
SD:	Standard deviation
SPSS:	Statistical package for the social sciences
ANOVA:	Analysis of Variance
Hb:	Haemoglobin
HCT:	Haematocrit
LPC:	leukofiltered packed cell.
WB:	Whole blood
S.K:	Serum Potassium
FDA:	Food and Drug Administration
Spp:	Species

TRALI:	Transfusion related acute lung injury
AHTR:	Acute haemolytic transfusion reaction
NA:	Not applicable

ABSTRACT

INTRODUCTION: Blood transfusion is an indispensable part of patient management, like all therapeutic procedures it has also some adverse effects. However, few complications of blood transfusion arise due to presence of leukocytes in donor's blood. Leukoreduction is the removal of leukocytes from blood components. Leukoreduction of components is a widely practiced and established strategy for reducing the incidence of transfusion associated adverse events.

AIM OF STUDY:- To evaluate the efficiency and quality of pre storage leukocyte reduction of whole blood following delayed storage at ambient temperature.

MATERIALS AND METHODS:- Blood was collected from voluntary blood donors into the blood bag with inline leucocyte filter for WB collection, The collected bags were divided into 3 groups of 30 units each.
Group A contain the indoor collected blood units into the blood bag with inline leucocyte filter for WB collection which will be kept for 4-6 hours at 4-6°C before filtration and separation, the leukocyte depleted red blood cell concentrate (RBCC) prepared from these units will be processed and stored at 4-6° C for 28 days.
Group B and Group C contain the collected blood units from local and distant camps and kept for 8-10 hours (Local camps) and 12-18 hours (Distant camps) that were stored and transported in cold box that kept the temperature under 10° C and kept at 4-6°C before filtration and processing, these leukocyte depleted RBCC will be stored at 4-6° C for 28 days.
Aseptic samples were assessed for complete blood count (Hb, RBC count, haematocrit, leukocyte), plasma Hb, RBC haemolysis, serum electrolytes (K+) and blood culture at pre and post filtration stages as well as during storage on fortnight basis thereafter.
Leucocyte count was checked with coulter counter method and then confirmed by peripheral blood film (PBF) and then rechecked by Neubauer chamber technique.

RESULTS:- Aseptic samples were assessed at fortnightly basis and shows that Hb levels vary between 14 to 20 g/dl, haematocrit between 45 to 64%, leukocyte count decreases from $6.43*10^3$/µL to $0.01*10^3$/µL, RBC count also decreases from $4.94*10^6$/µL to $2.45*10^6$/µL, plasma Hb increases from 12 to 87 mg/dL, haemolysis increases from 0.046 to 0.088%, potassium level increased from 3.74 to 31.93 mmol/L and blood culture shows no growth as well as only FNHTR and hypotension from two units were reported.

CONCLUSION:- Whole blood filtration prior to component preparation seems to be useful alternative method for obtaining leukoreduced RBC and plasma with minimal whole blood loss.

KEYWORDS:- Leukocytes, Leukoreduction, Febrile non haemolytic transfusion reaction, red blood cell concentrate.

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INTRODUCTION

Blood transfusion is an essential part of patient management. However, as with any therapeutic process, it has some side effects. However, a few difficulties of blood transfusion occur as a result of the presence of leukocytes in the donor blood. The improvement of techniques to lower the content of donor leukocytes has greatly reduced related adverse effects. White blood cells found in blood components have been linked to post-transfusion complications. These include primary HLAalloimmunization, which results in refractoriness to platelet transfusion[1], febrile non-haemolytic transfusion responses, and virus transmission, such as cytomegalovirus and human T cell lymphotropic viruses. Pre-storage leukodepletion has been proven to increase red blood cell quality preservation via leukoreduction.

The goal of this study is to clarify the biochemical and biophysical underpinnings that support the positive clinical outcomes associated with the use of leukodepleted erythrocyte units. Overall, the data reveal that leukodepletion not only selects red blood cells with a more durable plasma membrane, extending their viability, but also provides the appropriate amount of nutrients for a longer period of time.

WBC decrease in blood components can be accomplished by using commercially available leukocyte depletion filters. Recent research indicates that third-generation WBC reduction filters consistently achieve more than a 3 log10 reduction[2]. It is a critical component of patient management. However, the presence of leukocytes in donor blood causes only a small number of difficulties. The evolution ofprocedures for reducing the amount of donor leukocytes.

Leukoreduction refers to the process of removing leukocytes from blood components. Each leukoreduced product exhibited residual leukocyte counts less than $5*10^6$ cells per unit. Currently, leukoreduction is carried out utilizing leukocyte reduction filters, which capture leukocytes while allowing the desired blood product through.

Removing leukocytes below a threshold (<5*106) in a blood component can avoid alloimmunization and related hazards in these patients. Currently, the finest leukoreduction can be performed using third and fourth generation leukofilters, both in the laboratory and at the patient's bedside, as well as cutting-edge apheresis systems, however pre-storage leukoreduction is superior to bedside. [3]

Evidence suggests that WBC and platelet fragmentation occurs rapidly during storage, and that breakdown products such as histamine, elastase, acid phosphatase, and serotonin accumulate if WBC and platelets are not removed prior to storage. According to reports, pre-storage WBC reduction of red blood cell concentrates improves the units' storage characteristics, including reduced haemolysis, lower supernatant potassium, and higher post-storage 2,3-DPG and ATP levels. [4]The effect of duration, temperature, and filtration rate on the efficacy of WBC reduction was investigated in both controlled and paired donor formats. Blood products stored with additive solutions at varying temperatures play a key role in storage lesions. [3] The RBC membrane's cytoskeletal proteins were a significant source of damage. Reduced ATP and 2,3-DPG were reversible alterations resulting from the stored blood.

The objective of this study was to compare the efficacy of pre- and post-storage filtration. Filtration time, residual leukocyte count, RBC recovery, and haemolysis were all assessed after pre- or post-storage filtration. Post-storage filtration took much longer than pre-storage filtration, and haemolysis was significantly higher. The residual leukocyte counts and RBC recovery following post-storage filtering were similar to those obtained after pre-storage filtration. These findings suggest that pre-storage filtration is more efficient than post-storage filtration and may be responsible for the creation of higher-quality leukoreduction blood components. Beneficial effects of leukoreduction and pre-storage leukodepletion include the reduction of febrile non-haemolytic transfusion responses associated with CMV

transmission and anti-HLA vaccination, which leads to platelet refractoriness.

Recently, in-line filtering has been adopted, allowing for early WBC depletion of RBCC in closed systems before storage. However, comprehensive RBCC filtration necessitates centrifugation of blood bags and filters, which may result in lower centrifuge capacity and the need for precise cup packing. Whole blood filtration may increase the RBCC preservation properties while preserving plasma coagulation components. [5] In addition to comparing indoor and remote camp collections to see if differences in leukoreduction efficiency result in fewer acute transfusion reactions, our study will also look at the extent of haemolysis during processing and storage at different time intervals, as well as serum potassium levels.

AIMS AND OBJECTIVE

To evaluate the efficiency and quality of pre storage leukocyte reduction of whole blood, following delayed storage at ambient temperature.

REVIEW OF LITERATURE

Blood transfusion is a fundamental therapy in numerous pathological conditions. Many clinical reports describe adverse transfusion's drawbacks due to red blood cells alteration during storage.[6] Thus, the ability of the blood bank to increase the quality of erythrocyte concentrate units is critical to improving results and reducing transfusion responses.[6]All leukocytes are formed from common pluripotent hematopoietic stem cells through the action of numerous growth agents known as cytokines. Leukocytes are further classified into two types: granulocytes (neutrophils, basophils, and eosinophils) and agranulocytes (lymphocytes and monocytes). Cytokines act at multiple stages and in tandem to promote leukocyte proliferation, maturation, and function.[7]

Cytokines affect the development and maintenance of inflammation and immunity. The type of cytokine released by leukocytes is also determined by cellular interactions. The biological effects of cytokine release include fever, chills, rigors, and enhanced capillary permeability.[7]

Clinical investigations found a link between transfusions of red blood cells held for more than 14 days and an increase in infectious events, length of hospitalization, mechanical ventilation, multiple organ failure, and death. The cause of these bad effects is related to the morphological and metabolic changes that RBCs go through during prolonged storage, such as the creation of lactate, which causes a reduction in pH, depletion of adenosine triphosphate, and 2,3diphosphoglycerate. Storage at low temperatures (2-6 degrees Celsius) slows glycolysis, but it also reduces the activity of the Na+ K+ concentration in the conservation medium, increasing the danger of arrythmia in the event of a quick transfusion via a central vein. Storage is also known to cause changes in RBC shape and membrane loss. In this regard, the exact relationship between those storage defects and

the fate of the transfused cells remains uncertain, drawing the scientific community's interest.[8]

The formulation of the criterion for the selection of transfusion units is a hotly debated topic among clinical practitioners; the majority of opinion refers to the storage time of the bags. While there is evidence of a link between RBC storage time and morbidity/mortality in transfused patients, randomized controlled trials are now underway to confirm this finding. Given these considerations, reducing the RBS's storage duration would be an easy option. Methods for preserving the quality of RBCs during preservation are being investigated; the elimination of leukocytes prior to storage is one potential option.[6]

The favourable outcomes related with the introduction of RBC leukodepletion before storage are expected to result from the preservation of RBC quality for a longer period of time. Because the main difference between leukoreduced and leukodepleted RBC's is the significantly reduced number of leukocytes present in the bags of leukodepleted RBC's, progress in the optimization of RBC storage requires understanding how the presence of leukocytes influences RBC vitality, a condition that is currently associated with the maintenance of both proper shape and efficient metabolism.[6]

Pre-storage filtering of blood components that appears to be a useful strategy for reducing leukocyte-induced adverse responses and other problems. To establish whether it is better to filter whole blood before component separation, we compared the efficiency of in-line filtration of whole blood to that of post filtration.

The elimination of WBC from cellular blood components that has been linked to a reduction in a variety of transfusion-related adverse effects. These include fever, nonhemolytic transfusion responses, HLAalloimmunization with subsequent refractoriness to platelet transfusion, transfusion-associated GVHD, immunosuppression, and transfer of certain infectious leukotropic agents.[9]

Febrile non-haemolytic transfusion reaction (FNHTR), Rise in temperature of 1 degree Celsius or more above the pre transfusion baseline, in the absence of any underlying febrile illness. It usually occurs during or within few hours of transfusion and can be associated with chills, nausea, vomiting, tachycardia, hypertension. However asymptomatic rise in body temperature in a hypothermic patient to normal temperature is not FNHTR.[10]

Cytokine release is the primary cause of symptoms in FNHTR. There are two primary processes that lead to cytokine release. The first occurs when recipient antibodies and antigens engage with donor leukocytes. The antibodies target HLA and WBC-specific antigens detected on donor leukocytes.[11] The degree to which cytokines are released in response to antigen-antibody interactions also influences the severity of the relationship. the second method involves the infusion of produced cytokines and inflammatory mediators, which build in plasma-based blood components, particularly platelet-rich concentrate, during storage. These cytokines are released into the plasma following WBC preservation. The final stage in both pathways is to increase cytokine levels in the recipient, such as interleukin-1 beta, IL6, and tumour necrosis factor. Cytokines increase thermostatic set points in the hypothalamic thermoregulatory centre, causing fever.[12]

HLAAlloimmunization: A delayed immunological consequence of blood transfusion. It is also a primary cause of platelet refractory condition. HLA class 1 antigens are involved in primary alloimmunization and can be found in blood components in three forms: intact cells, degenerating cells and cell fragments, and soluble plasma lipoproteins. The development of anti-HLA antibodies during alloimmunization is one of the leading causes of platelet refractoriness and inability to increase platelet count following platelet transfusion, particularly in the patients with hematologic malignancies.[13]

Transfusion-Associated Graft versus Host Disease (TA-GVHD): It occurs when the donor T lymphocytes react to proteins on host cells, multiplying and targeting host organs, especially the liver, skin, and intestinal tract. It is most commonly associated with whole blood or red cell transfusions, although it can also occur with fresh frozen plasma and platelet concentrates if viable lymphocytes are present.[12]

Indications and standards for transfusing leukoreduced blood components are not standardized or widely acknowledged. This is primarily owing to the high cost of leukoreduction surgeries. Some governments have decided for universal leukoreduction, whereas others believe further study is needed to justify the costs.[12]

Leukocyte filter: A typical filter design consists of a sterilizable fibre material, upstream layers that prevent clotting, a plastic housing, and inlet/outlet ports that prevent air bubble retention, as well as the possibility of a tight seal between filter layers and housing to prevent channelling.[14] There are two types of filtration mechanisms: surface/screen filtration and deep filtration. Depth filters use compressed wool fibres placed irregularly, whereas screen filters use fibres stacked in several layers in a regular pattern.[15] Screen filtration restricts particle separation to the filter surface, whereas depth filtration does not. In depth filtration, most often used mechanism for leukocyte reduction, the porous structure has a wide variety of pore sizes across the filter matrix, allowing particles to be retained at any point within the filter. Leukocytes are captured in filters through mechanical sieving, direct adhesion, and cell-cell contact.

Leukofiltration can be performed at the time of collection and processing, after processing (at the blood bank), or at the patient's bedside (post storage). They each have their own set of perks and downsides. However, pre-storage leukoreduction is now the most frequently acknowledged technique. The advantages of pre storage over post storage leukoreduction are as follows:

- It eliminate the scope of inflammatory (interleukins-1, interleukins-6, tumour necrosis factor) cytokine accumulation due to leukocytes, during storage and therefore it is quite efficient in the prevention of febrile non haemolytic transfusion reactions.[16-18]
- It also reduces the risk of HLAalloimmunization in multi transfused patients as it also removes the intact leukocytes as against filtration at the bedside and here leukocyte fragments after storage can pass through filters and allo immunize the recipient against donor antigens.[19-20]
- Pre storage leukofiltration can also reduces the risk of leukotropic virus transmission as leukocytes help in disintegrate and release the intracellular organism after 72 hours of storage in the blood components.[21-22]
- It is always easier to perform leukocyte quality control in the laboratory rather than by the patients bedside, hence during pre storageleukoreduction blood components can be thoroughly studied and evaluated for quality control and various factors affecting the process of leukofiltration modified accordingly.[23-24]

Because of the complicated physical and biologic processes involved in leukocyte retention by the filter medium, various parameters influence the degree of leukocyte reduction, including filter capacity, leukocyte input, filtration temperature, and many more. The contribution of each filtration mechanism that is determined by the type of fibres, the physical qualities of blood cells, the mix of blood components, and the filtering process.[9] Critical stages in the red cell filter filtration process include leukocyte and platelet numbers, the presence of plasma, temperature, product age, flow, priming, and washing.[25] The temperature of filtration had a significant impact on the performance of leukocyte filtration of RBC. Almost every study has revealed that filtering efficiency improves when the operation is performed at refrigerated temperatures.

To enable standardization of all blood units, the collected WB units are immediately placed on special 1,4butanediol cooling trays and kept at room temperature until processing.[26]

Patients on regular transfusion therapy, such as thalassemia major patients, can benefit from the packed red cell selective pre storage leukofiltration policy, which requires skillful inventory management and active coordination between the transfusion therapy clinic and the blood bank. Platelet concentrates can be pooled and leukofiltered for a specific group of haemato-oncology patients in blood centers prior to storage. This technique can achieve 3-4 log leukoreduction, but it requires devoted technical manpower for tight quality control, skilled inventory management, and active coordination with the treating units.[25]

Harvesting blood components using apheresis technology: they are typically 3-4 log leukoreduced and provide greater therapeutic benefits than random donor products. This could be quite beneficial to individuals who are refractory to platelet transfusion with an HLA-matched donor. However, the expense of consumables limits its utility in general, although it remains the best alternative for those who can afford it.[26]

Storage of red blood cells in preservative media causes severe metabolic, biochemical, and molecular alterations to erythrocytes, which are commonly referred to as "storage lesions".[27,28] Blood products, such as RBC, that are stored with additive solutions at various temperatures contribute greatly to storage lesions.[28] The most likely locations of damage will be cytoskeletal proteins in the RBC membrane.[29] These membrane alterations will cause RBCs to become brittle, increasing osmotic fragility and electrolyte imbalance. Great efforts have been made to produce a suitable and safe supply of blood, with more benefits than drawbacks. Currently, the storage techniques for blood bags in blood banks require certain parameters to

provide the maximum storage time for a healthy and safe blood supply. Blood bags can now be stored for up to 42 days at 2-6°C, as long as the average haemolysis is less than 0.8% and more than 75% of red blood cells survive the first 24 hours following transfusion.[30]However, pathogenic effects might occur in the stored blood, which are known as storage lesions. Storage lesions are considered to reduce the efficiency of stored blood and their capacity to play their needed role following transfusion, although these hypotheses have no clear evidence yet. [31] Although storage lesions in preserved red blood cells are now widely recognized, there are still structural and functional characteristics of stored red blood cells that must be explained. In this investigation, we are particularly interested in observing progressive irreversible changes in RBC shape and correlating them to osmotic fragility changes throughout storage duration. We also aim to link the observed haematocrit changes with morphological changes.

Red blood cell transfusions are used to improve haematocrit levels in patients with anaemia or to replenish volume after an acute bleeding episode, but the primary issues with packed red cell preservation in blood banks are haemolysis and morphological changes of RBC with time.

The quality of red cells held for 5-6 weeks is determined by the kind of anticoagulant, the first handling, processing during component separation, the composition of the additive solution, and the nature of the storage conditions inside the transfusion service.[32,33] When blood is mixed with an anticoagulant solution and stored at 4 °C, red cells change shape from discs to echinocytes and then to spheres, become more rigid, shed lipid, exhibit various biochemical changes, particularly a decrease in ATP and DPG content, and gradually lose their ability to survive in the circulation after transfusion. These consequences are caused by the paralysis of red cell membrane function, which includes the membrane-associated Na+/K+ pump.[34]

The storage system's goal is to protect the metabolic machinery and membrane from damage while also maintaining ATP and adenosine concentrations to increase post-transfusion survival. Other substances with possible benefits include inorganic phosphate (both as a substrate for the maintenance of organic phosphate molecules and as a buffer) and citrate (for anticoagulant buffering and affecting intracellular pH).[35,36]Mannitol (and sorbitol) have been used to provide osmotic support and to prevent spontaneous storage-associated haemolysis. Anticoagulants used for the purpose include ACD (adenine, citrate, and dextrose), CPD (citrate, phosphate, and dextrose), and CPDA-1 (citrate, phosphate, dextrose, and adenine).[37] The most frequent additive solution for RBC storage is SAG-M (Saline, Adenine, Glucose, and Mannitol).[36]

SAG-M is an additive solution that not only extends the shelf life of packed red cells, but also lowers haemolysis and vesiculation, most likely because its free radical scavenger and membrane stabilizer properties.[36]

In last years, there has been an emphasis on stricter control over the quality of blood and its derivatives. The level of haemolysis is one such quality measure for preserved red blood cells. Detecting excess haemolysis from component processing and the storage has significant implications for the transfused patient.[38] Leukocytes emit the variety of substances and enzymes, particularly proteases, which contribute considerably to an increase in red cell haemolysis during storage. As a result, commercially available leukocyte reduction filters reduce the rate of haemolysis in stored red cell units. Despite the increased use of additive solutions and all of the precautions adopted for red cell storage, some haemolysis is unavoidable.[38,39] The amount of haemolysis can be evaluated using estimated by various techniques like visual assessment, spectrophotometric assays (TMB method), photometric method, and microplate technique.[39,40]

Transfusion of packed RBCs is a life-saving technique that enables complex surgeries, organ and bone marrow transplantation, myelotoxicchemotherapy, and recovery from significant blunt and

piercing injuries. In most countries, the shelf life of RBC concentrates is limited to 42 days in order to maintain 24-hour recoveries over 75% and haemolysis below 0.8%. During storage, red blood cells accumulate metabolic compounds known as the "storage lesion".[41] The storage lesion impairs cation homeostasis, accumulates reactive oxygen species (ROS), alters proteomic profiles, and causes progressive vesiculation of membrane portions that accompany the loss of discocytic morphology in exchange for a spheroechinocytic RBC morphology.[42-45] These results cleared the way for exploratory research into the impact of various storage solutions (e.g., antioxidant, vitamin C, N-acetyl cysteine, or serotonin supplementation) or techniques (e.g., anaerobic storage) on stored RBC metabolic profiles. Recent advances in metabolomics include the use of ultra HPLC separations coupled with online mass spectrometry detection (UHPLC-MS), the creation of publicly accessible metabolome databases, and the implementation of software for mining and interpreting extremely large data sets.[46]

Furthermore, bioactive compounds like as histamine, lipids, and cytokines released by "passenger" leucocytes may have a direct effect on metabolic and physical changes associated with senescence in cells and are linked to RBC storage medium lesion.[47] Other data suggests that hypothermic storage of red blood cells may result in lower metabolism and energy requirement, rendering the ATP-dependent sodium potassium pump inactive and allowing sodium and potassium to freely flow into and out of cells.[48] According to current research, RBC hypothermic storage lesion is responsible for the link between blood transfusion and an increased duration of stay in the hospital, higher infections, multiple organ system failure, and, ultimately, increased morbidity and mortality.[49]

Hyperkalaemia is defined as a serum potassium level over the reference range, with arbitrary criteria (>5.00, >5.50, or >6.00 mmol/L) used to denote severity.[50] Patients with chronic and severe renal disease may be at significant risk of elevated plasma potassium if certain comorbid disorders and other factors that interfere with kidney

potassium excretion are present. Any increase in serum potassium levels in persons with severe hyperkalaemia, particularly in critically ill cases, can lead to serious consequences and even death.[51]

The major electrolyte in the extracellular fluid (ECF) is sodium which has about 98% of its total quantity in the ECF, and only about 2% is found in the intracellular fluid (ICF).

The effect of stored blood transfusion on the recipient's potassium or sodium levels as well as acid-base balance is complex. It is, however, highly reliant on the volume of blood transfused, the rate of transfusion, the rate of citrate metabolism, and the patient/recipient's fluctuating peripheral perfusion.[52] Failure to detect apparent electrolyte abnormalities has been found to be lethal in some cases.

The impact of banked blood transfusions on the recipient's potassium and sodium levels, as well as acid-base balance, is complex. It is, however, extremely dependent on the volume of blood transfused, the rate of transfusion, the rate of citrate metabolism, and the patient/recipient's variable peripheral perfusion.[52] Failure to notice obvious electrolyte abnormalities has been proved to be fatal in some circumstances.

Blood and blood components are potential sources of infection for a wide range of known and undiscovered pathogens. Testing for transfusion-transmitted illnesses has reduced viral transmission among the individuals who have received the blood transfusions. Understanding the prevalence and causes of bacterial infection in blood transfusions is critical. It aids in the development of preventive measures at blood transfusion centres as well as in the reduction of transfusion-transmitted bacterial illnesses. The characterization of bacterial isolates and the types of contaminated blood components is critical for public health, having infections has decreased viral transmission among individuals who have received blood transfusions.[53] However, the low but certain danger of bacterial contamination has arisen as a residual but significant concern to transfusion-transmitted illnesses.

An unfavourable reaction or event is an unwanted response or impact in a patient that is temporally related to the administration of blood or blood components.[54] Even in affluent countries, non-infectious transfusion complications are the leading cause of morbidity and mortality.[55] Non-infectious adverse transfusion reactions (NIATRs) are the terms used in this review to describe non-infectious adverse events associated with blood transfusions. As an aid to help the clinicians in a suspected NIATR, an approach to differential diagnosis is being provided below in the diagram:

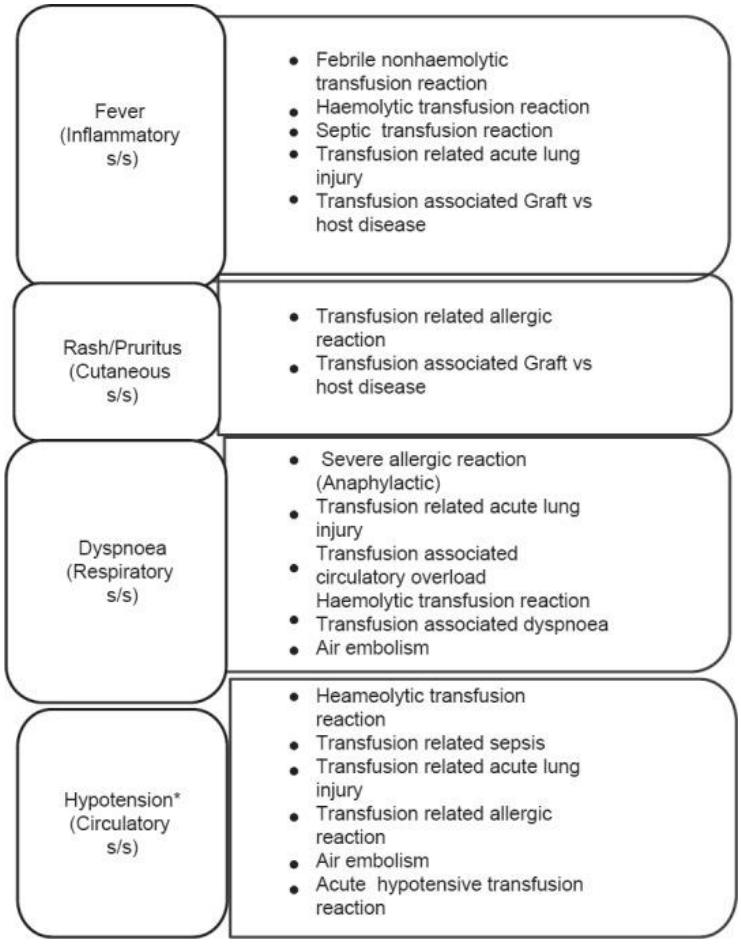


FIGURE 1: S Differential diagnosis of adverse transfusion reactions depicts the clinical presentation (signs and symptoms).[74]

The American Association of Blood Banks technical manual provides recommendations for the recognition, diagnosis, investigation, and classification of non-infectious transfusion responses, making it a

useful resource for doctors and other health care workers working with blood transfusions.[56]

Acute transfusion responses are responsible for the majority of significant adverse reactions and occurrences. A better prognosis can be achieved by being aware of the numerous clinical signs of acute transfusion responses and being able to assess significant reactions in a timely manner. Observation and monitoring are necessary during the transfusion session, particularly within the first 15 minutes. A standard operating procedure should include specifics for documenting, reporting, evaluating, and following up on all adverse events. The evidence-based approach of "restrictive strategy" or "conservative approach" of blood transfusion to limit the amount of unnecessary transfusions has resulted in a significant shift in current clinical practice.[57]

MATERIAL AND METHODS

Blood units were collected from voluntary blood donors into the blood bag with inline leucocyte filter for WB collection, that contain 63 ml of citrate phosphate dextrose (CPD) anticoagulant and saline adenine glucose mannitol (SAGM) as RBC preservative solution. Collected whole blood centrifuged using a heavy spin 3600 RPM at 15 minutes with a temperature setting of 4°C. place the primary bag containing centrifuged blood on a plasma extractor, release the spring allowing plate of the extractor to contact the bag. Clamp the tubing between the primary and satellite bags with a haemostat or if mechanical sealer not used than make a loose knot in the tubing. If two or more satellite bags attached than apply the haemostat to allow plasma to flow into only one satellite bag, remove the appropriate amount of plasma to obtain the desired haematocrit. Reapply the haemostat when the desired amount of supernatant plasma has entered the satellite bag, seal the tubing between the primary bag and satellite bag in two places also check that the satellite bag has same donor number as that on the primary bag and cut the tubing.

Blood bag with inline leucocyte filter for WB collection and separation of two different blood components (leukodepleted RBC's and leukodepleted plasma) are used. The primary bag contains CPD solution and is attached to WB/PR filter. One satellite bag comes with SAGM-2 solution for red cell preservation.

The collected blood units were divided into 3 groups of 30 units each.

Blood bag with inline leucocyte filter for WB were used and divided into the three groups.

Group A contains the indoor collected blood units in Blood bag with inline leucocyte filter for whole blood which was kept for 4-6 hours at 4-6° C before filtration and separation, the leukocyte depleted red blood cell concentrate (RBCC) prepared from these units were processed and stored at 4-6°C for 28 days.

Group B contains the collected blood units from the local camps that were stored and transported in cold box that kept the temperature under 10° C and these collected units were kept for 8-10 hours at 4-6°C before filtration and separation and the leukocyte depleted red blood cell concentrate (RBCC) prepared from these units were processed and stored at 4-6° C for 28 days.

Group C contains the collected blood units from the distant camp and that was stored and transported in cold box that kept the temperature under 10° C, these collected units were kept for 12-18 hours at 4-6° C before filtration and separation, the leukocyte depleted red blood cell concentrate (RBCC) prepared from these units were processed and stored at 4-6° C for 28 days.

Aseptic samples were assessed for complete blood count (Hb, RBC count, haematocrit, leukocyte), plasma Hb, RBC haemolysis, serum electrolytes (K+) and blood culture at pre and post filtration stages as well as during storage on fortnight basis thereafter.

Leucocyte count was checked with coulter counter method and then confirmed by peripheral blood film (PBF) and then rechecked by Neubauer chamber technique.

SOURCE OF DATA

The study was conducted in the Immunohaematology and Blood Transfusion department and pathology department of Dayanand medical college and hospital.

METHOD OF COLLECTION OF DATA

INCLUSION CRITERION

- 450±10% ml of blood collected in quadruple bag with inline filter for whole blood.

EXCLUSION CRITERION

- Over volume bag
- Reactive units
- Insufficient amount collected units

DURATION OF STUDY

One year, 1st January, 2023 to 31st December, 2023

OUTCOME

Whole blood filtration prior to component preparation seems to be useful alternative method for obtaining leukoreduced RBC and plasma with minimal whole blood loss and may also reduce adverse reactions in transfused patients.

STATISTICAL ANALYSIS

Data will be described in terms of range; mean ±standard deviation (± SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. To determine whether the data is normally distributed, a Kolmogorov-Smirnov test will be used. Comparison of quantitative variables between the study groups will be done using ANOVA and Kruskal-Wallis Test with post hoc Tukey's for independent samples for parametric and non-parametric data respectively. For comparing categorical data, Chi square ($\chi 2$) test will be performed and fisher exact test will be used when the expected frequency is less than 5. A probability value (p value) less than 0.05 will be considered statistically significant. All statistical calculations will be done using (Statistical Package for the Social Science) SPSS 21version (SPSS Inc., Chicago, IL, USA) statistical program for Microsoft Windows.

OBSERVATIONS AND RESULTS

In this study we have taken 90 blood units from random donors in quadruple bag with inline filter for whole blood and divided into 3 groups of 30 units each. After collection, processing and storage, samples will be assessed for complete blood count (Hb, RBC count, haematocrit, leukocyte), plasma Hb, RBC haemolysis, serum electrolytes (K+) and blood culture at pre and post filtration stages as well as during storage on fortnightly basis thereafter.

TABLE 1: COMPARISON of haemoglobin levels in indoor collection, local and distant camps

	Indoor		Local camp		Distant camp		F	p-value
	Mean	SD	Mean	SD	Mean	SD		
Pre_HB (g/dL) (WB)	14.65	1.47	13.85	1.50	13.69	1.48	3.596	0.032
14TH DAY Post_HB (g/dL) (LPC)	17.48	2.41	17.18	2.42	17.81	2.40	0.519	0.597
28TH DAY Post HB (g/dL) (LPC)	19.89	1.77	18.97	2.47	19.82	1.65	1.968	0.146

The Hb level was 14.65,17.48 and 19.89 (g/dl) on days 0, 14, and 28 in indoor collection. In local camps, it was 13.85, 17.18 and 18.97(g/dl), while in distant camps, it was 13.69, 17.81 and 19.82 (g/dl). The range is between 14 and 20 g/dl as showing in this table.

TABLE 2: COMPARISON OF HAEMATOCRIT IN INDOOR COLLECTION, LOCAL AND DISTANT CAMPS

	Indoor		Local		Distant		F	p-value
	Mean	SD	Mean	SD	Mean	SD		
Pre_HCT (%) (WB)	45.76	3.86	43.01	5.32	43.26	4.59	3.251	0.044
14TH DAY Post HCT (%) (LPC)	56.27	7.64	54.48	8.00	56.61	6.50	0.715	0.492
28TH DAY Post_HCT (%) (LPC)	63.63	5.93	60.83	7.94	62.65	5.82	1.371	0.259

The above table shows that on days 0, 14, and 28 for indoor collection, the average HCT level of blood unit was 45.76%, 56.27%, and 63.63%; In local camp. It was 43.01%, 54.48%, and 60.83%; and in distant camp it was 43.26%, 56.61%, and 62.65%. The p value at day 0 of collection was 0.044 and was significant indicating that there was less variation in the haematocrit levels, indicating that it was safe to use.

TABLE 3: COMPARISON OF LEUCOCYTE COUNT IN INDOOR COLLECTION, LOCAL AND DISTANT CAMPS

	Indoor		Local Camp		Distant Camp		F	p-value
	Mean	SD	Mean	SD	Mean	SD		
Pre_LEUCOCYTE (10^3/µL) (WB)	6.43	0.96	6.36	1.53	6.43	1.40	0.027	0.973
DAYPost_LEUCOCYTE (10^3/µL) (LPC)	0.01	0.01	0.02	0.03	0.01	0.01	2.185	0.119
28TH DAY Post_LEUCOCYTE (10^3/µL) (LPC)	0.01	0.01	0.01	0.02	0.01	0.01	0.341	0.712

The leucocyte count of whole blood in indoor collection as well in local and distant camps was 6.43, 6.36, 6.43 (10^3/µL) at 0 day of collection that reduced to 0.01 post filtration upto 28th day. The leucocyte count was lower during post filtration that leads to lower log drop in WBC contents.

TABLE 4: COMPARISON OF RBC COUNT IN INDOOR COLLECTION, LOCAL AND DISTANT CAMPS.

	Indoor		Local camp		Distant camp		F	p-value
	Mean	SD	Mean	SD	Mean	SD		
Pre RBC (10^6/µL) (WB)	4.94	0.50	4.93	0.71	4.87	0.68	0.086	0.917
14TH DAY Post _RBC (10^6/µL) (LPC)	2.92	0.82	3.13	0.86	3.17	0.75	0.810	0.448
28TH DAY Post RBC (10^6/µL) (LPC)	2.45	0.73	2.74	0.80	2.88	0.72	2.533	0.085

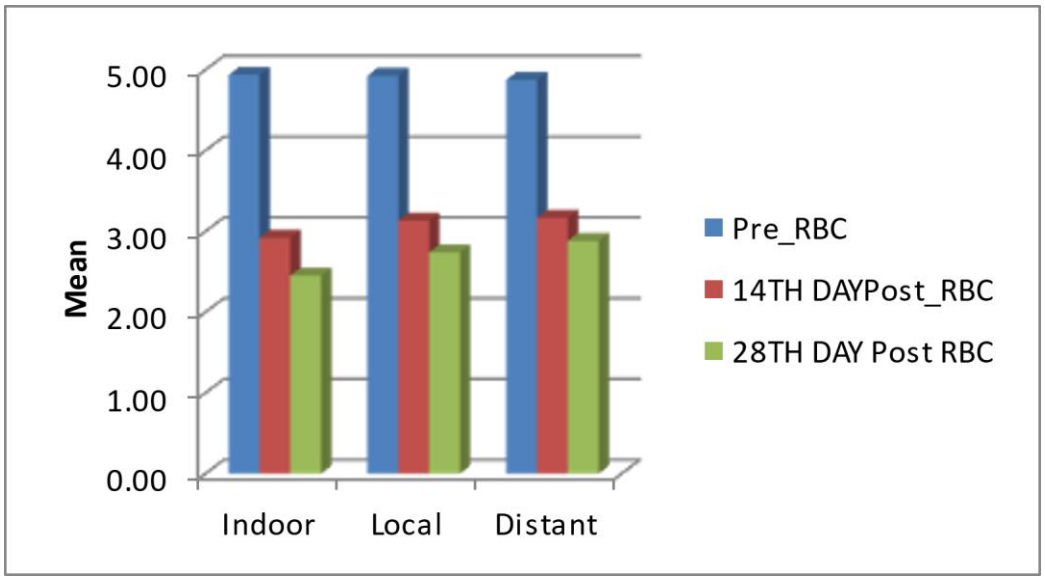


FIGURE 2: COMPARISON OF RBC COUNT IN INDOOR COLLECTION, LOCAL AND DISTANT CAMPS

The RBC counts at 0, 14, and 28 days in an indoor collection were 4.94, 2.92, and 2.45 (10^6/µL), whereas the count in local camps were 4.93, 3.13, and 2.74 (10^6/µL) and the counts in distant camps were 4.87, 3.17, and 2.88 (10^6/µL). p value at 0 day of collection was 0.917 whereas at 14th and 28th day of collection it was 0.448 and 0.085.

TABLE 5: COMPARISON OF PLASMA HAEMOGLOBIN LEVEL IN INDOOR COLLECTION, LOCAL AND DISTANT CAMPS

	Indoor		Local Camp		Distant Camp		F	p-value
	Mean	SD	Mean	SD	Mean	SD		
Pre_plasmaHb (mg/dL) (WB)	12.23	3.22	12.87	2.96	13.87	3.49	1.949	0.149
14TH DAY Post_plasmaHb (mg/dL) (LPC)	27.70	11.10	38.47	19.08	54.33	13.99	23.659	0.001
28TH DAY Post_plasmaHb (mg/dL) (LPC)	42.23	8.45	50.83	19.24	87.53	35.44	30.689	0.001

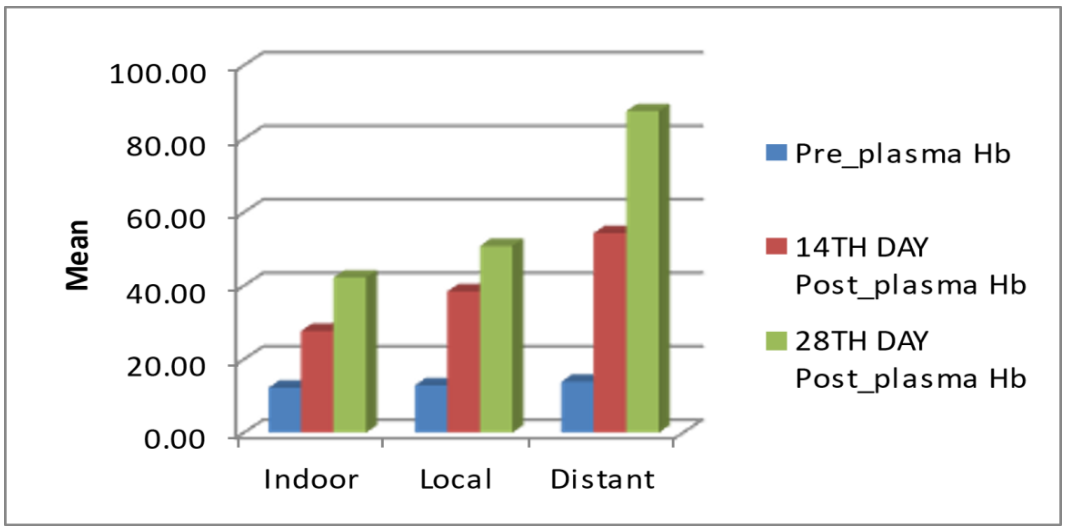


FIGURE 3: COMPARISON OF PLASMA HAEMOGLOBIN LEVEL IN INDOOR COLLECTION, LOCAL AND DISTANT CAMPS

The above table shows the change in the average plasma haemoglobin level from day 0 to day 28 of storage. The plasma haemoglobin level increased during storage, rising from 0 to the 28th day. There is variation in the range of 12.23 to 42.23 mg/dl for indoor collection, 12.87 to 50.83 mg/dl for local camps, and 13.87 to 87.53 mg/dl for distant camps. Since the plasma was separated before being stored and processed, Day 0 value was used as a control for statistical analysis. Significant level was observed on day 14^{th} and day 28^{th}.

TABLE 6: COMPARISON OF % HAEMOLYSIS IN INDOOR COLLECTION, LOCAL AND DISTANT CAMPS.

	Indoor		Local Camp		Distant Camp		F	p-value
	Mean	SD	Mean	SD	Mean	SD		
%HEMOLYSIS (WB)	0.046	0.014	0.054	0.017	0.058	0.016	4.810	0.010
14TH DAY %HEMOLYSIS (LPC)	0.071	0.034	0.107	0.065	0.133	0.043	12.425	0.001
28TH DAY %HEMOLYSIS (LPC)	0.078	0.022	0.110	0.060	0.170	0.088	16.989	0.001

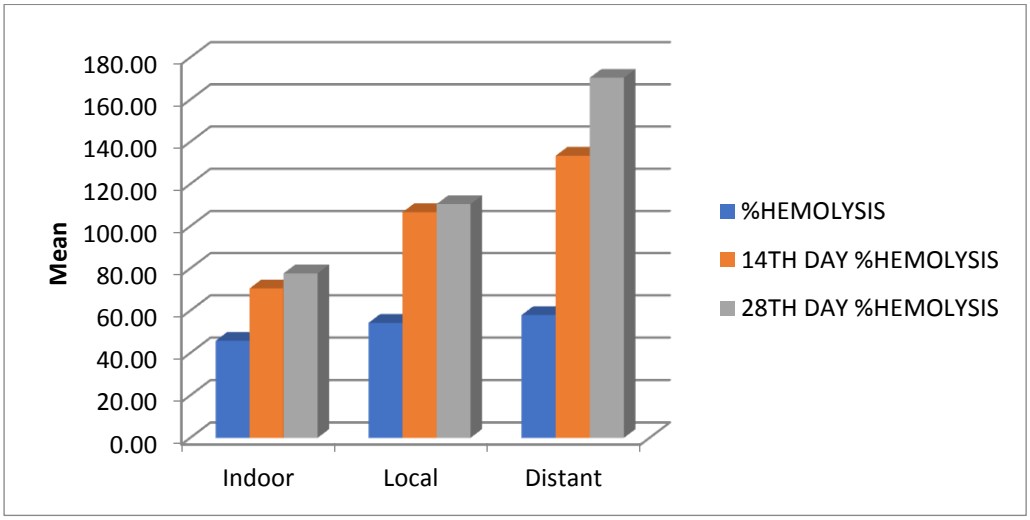


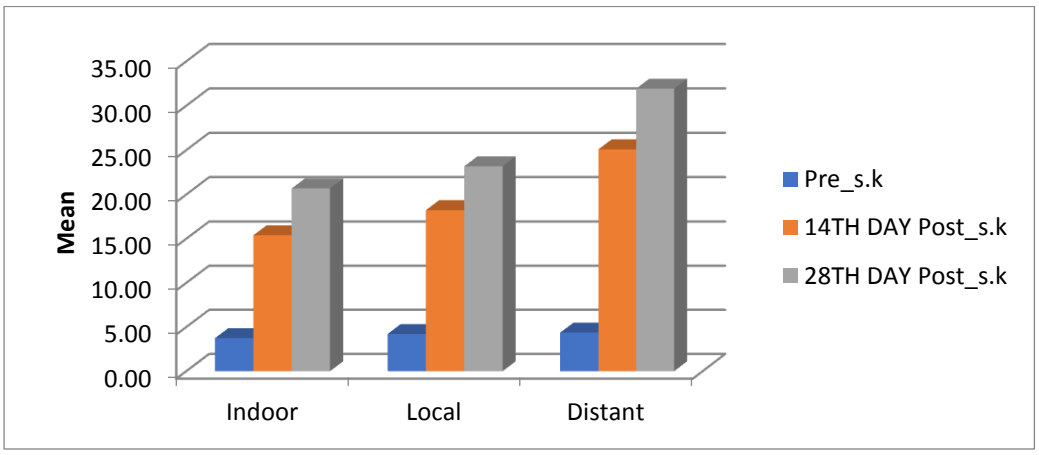
FIGURE 4: COMPARISON OF % HAEMOLYSIS IN INDOOR COLLECTION, LOCAL AND DISTANT CAMPS

The above table shows the percentage of haemolysis from the first to the 28th day of storage in both local and distant camps as well as in indoor collection. The percentage of haemolysis on day 0 of indoor collection was 0.046%; following processing and component separation, this percentage increased to 0.078%. Up to day 28, there was still an increase in haemolysis percentage with storage. The same outcomes were observed in distant camps and local camps, where the

percentage of haemolysis increased from 0.054% on day 0 to 0.110% on day 28. Additionally, in the distant camp, it rose from 0.058% on day 0 to 0.170 on day 28. The overall mean haemolysis ranges from 0.010 to 0.001 which was much below the permissible level of 0.8% haemolysis according to the European guidelines and 1% limit according to FDA. So the significant levels were observed and blood was safe to use.

TABLE 7: COMPARISON OF POTASSIUM LEVELS IN INDOOR COLLECTION, LOCAL AND DISTANT CAMPS

	Indoor		Local camp		Distant camp		F	p-value
	Mean	SD	Mean	SD	Mean	SD		
Pre_s.k (mmol/L) (WB)	3.74	0.54	4.22	0.66	4.37	0.89	6.334	0.003
14TH DAY Post_s.k (mmol/L) (LPC)	15.39	3.88	18.24	1.84	25.09	5.67	44.226	0.001
28TH DAY Post_s.k (mmol/L) (LPC)	20.69	3.98	23.19	2.83	31.93	6.73	45.353	0.001



The serum potassium levels throughout all samples, collected on days 0, 14, and 28 in indoor collection, local and distant camps, began at 3.74 mmol/l, which is a normal value. However, on day 28 in indoor collection, there was a significant increase to 20.69 mmol/l. In nearby camps, it ranged from 4.22 to 23.19 mmol/l, whereas in distant camps, it ranged from 4.37 to 31.93 mmol/l. These measurements, which were made both before and after filtration, demonstrate how potassium levels rise when storage times are extended. The rise in potassium level was statistically significant from day 0 of collection to 28th day of collection and storage.

FIGURE 5: COMPARISON OF POTASSIUM LEVELS IN INDOOR COLLECTION, LOCAL AND DISTANT CAMPS

TABLE 8: COMPARISON OF BLOOD CULTURE IN INDOOR COLLECTION, LOCAL AND DISTANT CAMPS

		Indoor		Local		Distant		Total
		No.	%age	No.	%age	No.	%age	
Blood culture	No growth	30	100.0%	30	100.0%	30	100.0%	90
Total		30	100.0%	30	100.0%	30	100.0%	90

After two weeks of incubation, this table demonstrates that there has been no growth in both nearby and far-off camps as well as indoor collections.

TABLE 9: COMPARISON OF ADVERSE DONOR EVENT IN INDOOR COLLECTION, LOCAL AND DISTANT CAMPS

		Indoor		Local		Distant		Total	**Chi-square value**	p-value
		No.	%age	No.	%age	No.	%age			
Adverse reaction	fever	0	0.0%	0	0.0%	1	3.3%	1	6.069	0.416
	hypotension	0	0.0%	1	3.3%	0	0.0%	1		
	NO	30	100.0%	29	96.7%	28	93.3%	87		
	rashes	0	0.0%	0	0.0%	1	3.3%	1		
Total		30	100.%	30	100.%	30	100.%	90		

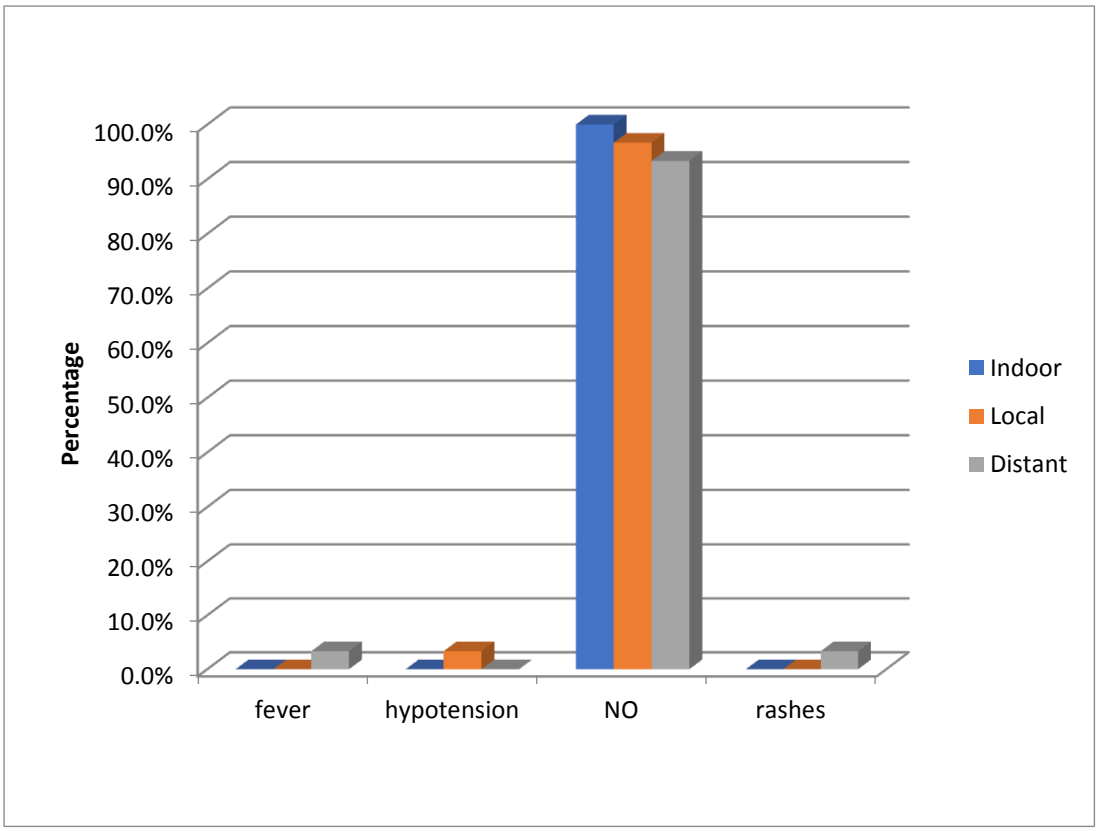


FIGURE 6: COMPARISON OF ADVERSE DONOR EVENT IN INDOOR COLLECTION, LOCAL AND DISTANT CAMPS

The above data shows that no adverse donor event reported from any blood unit during indoor collection on days 0, 14, or 28. An adverse donor event FNHTR (rash, fever) was reported from the blood unit from the distant camp collection, in addition to the hypotension from the local camp collection. The longer time required for component preparation and storage could be the cause of this.

DISCUSSION

Leukoreduction is the removal of leukocytes from blood components. It is a widely practiced and established strategy for reducing the incidence of many transfusion reactions, including the most common febrile non haemolytic transfusion reaction. Overall, the data show that leukodepletion not only selects red blood cells with a more durable plasma membrane, extending their viability, but also supplies the necessary nutrients for a longer period of time.However, the presence of leukocytes in donor blood causes only a few complications. There are numerous methods of leukoreduction and these have improved with technological advancement. The current most accepted and efficient method of leukoreduction is through use of leukocyte filters.

Haemoglobin and haematocrit level: -

In our study there is difference in the effect of storage duration on haemoglobin and haematocrit levels on day 0 to 28th day of storage.

The average haemoglobin level on day 0,14th and 28th was 14.65 g/dL, 17.48 g/dL and 19.89 g/dL (indoor) whereas it was 13.85 g/dL, 17.18 g/dL and 18.97 g/dL (local camp) and 13.69 g/dL, 17.81 g/dL and 19.82 g/dL (distant camp). This result is comparable with Sonya harisanti et al,[58]study in which pre filtration haemoglobin was 55.38 g/dL and post filtration haemoglobin was 59.72 g/dL. RiniAstuti et al [59]study and Naim et al [60]study also showed the increase in the haemoglobin level with the increasing storage time period. According to Riswanto et al[61]study Changes in haemoglobin levels towards the storage time in the PRC showed fluctuations.

The average haematocrit level on day 0, 14th and 28th was 45.76%, 56.27% and 63.63% (indoor) whereas it was 43.01%, 54.48% and 60.83% (local camps) and 43.26%, 56.61% and 62.65% (distant camps). The results are comparable with Sonya harisanti et al,[58]studyin which pre filtration haematocrit was 69.4% and post filtration was 74.00% respectively. The findings of the current study are consistent with previous studies by Karon et al., and Spinelli et al.,

which proved that an increase in free Hb and F2α-isoprostane levels occurred during PRBC storage.[62]

TABLE 10:- COMPARISON OF HB AND HCT WITH ANOTHER STUDY

Parameters	SONYA HARISANTI ET AL [58]	**PRESENT STUDY**
Pre Hb (g/dl) (WB)	55.38	14.65
Post Hb (g/dl/) (LPC)	59.72	19.89
Pre HCT (%) (WB)	69.4	45.76
Post HCT (%) LPC	74	63.63

Leucocyte and RBC count: -

In our study Leucocyte counts for whole blood on 0,14th and 28th day was 6.43*10^3/µL (indoor), 6.36*10^3/µL (local camps) and 6.43*10^3/µL (distant camps). Post filtration average leucocyte count was 0.01*10^3/µL in indoor collection as well as in local and distant camps. Similar results found in the JD Smith et al[63]studies in which pre filtration leucocyte count was 2.61*10^9 leucocyte/unitsthat decreases to 0.62*10^9 leucocyte/units duringpost filtration. Also, Eilat Shinar et al[64]study shows pre filtration leucocyte count was 2.52*10^9 leucocyte/units and post filtration show 0.69*10^9 leucocyte/units. The leucocyte count was lower during post filtration that leads to lower lower log drop in WBC contents.

The RBC count on day 0,14th and 28th day was 4.94*10^6/µL, 2.92*10^6/µL and 2.45*10^6/µL (indoor) whereas in local camp it was 4.93*10^6/µL, 3.13*10^6/µL and 2.74*10^6/µL and in distant camp it was 4.87*10^6/µL, 3.17*10^6/µL and 2.88*10^6/µL. This result is comparable to to Muhammad Sardar M et al study[65], in which pre-RBC count is 3.24 *10^6/µL that decreases to 2.62 *10^6 /µL during post filtration.

TABLE 11:- COMPARISON OF LEUKOCYTE COUNT AND RBC COUNT WITH OTHER STUDIES

Parameters	JD SMITH ET AL[63]	EILAT SHINAR ET AL[64]	MUHAMMAD SARDAR M ET AL[65]	**PRESENT STUDY**
Pre leukocyte (10^3/µL) (WB)	2.61	2.52	NA	6.43
Post leukocyte (10^3/µL) (LPC)	0.62	0.69	NA	0.01
Pre RBC (10^6/µL) (WB)	NA	NA	3.24	4.94
Post RBC (10^6/µL) (LPC)	NA	NA	2.62	2.45

Percentage haemolysis and plasma haemoglobin levels: -

In our study the proportion of haemolysis during 0,14th and 28th day was 0.046%, 0.071%, 0.078% (indoor) whereas in local camp it was 0.054%, 0.034% & 0.110% and in distant camp it was 0.058%, 0.133% and 0.170%. The percentage of haemolysis increased with storage up until day 28. According to Sayeedul Hasan et al[66]study mean haemolysis on day 1 was 0.081% that rose to 0.297% upto day 28th. Also, similar results shown by M.I.CGyongyossy-Issa et al[67]study i.e haemolysis at day 1 was 0.04 that rose 0.07% with increasing storage period. However, the extent of haemolysis did not exceed the permissible limit for haemolysis of 0.8% up to 28 days of storage.

Our study shows how the average plasma haemoglobin level changed between the first day of storage and day 28. During storage, the plasma haemoglobin level rose from day 0 to day 28. The ranges for indoor collection are 12.23 to 42.23 mg/dl, local camps are 12.87 to 50.83 mg/dl, and distant camps are 13.87 to 87.53 mg/dl. Similar results were shown by Sayeedul Hasan et al [66], i.e on day 1 mean plasma Hb level was 23.8 mg/dl that rose to 149.9 mg/dl whereas Sawant et al. study[68] and Mukherjee et al study[69]also shows that there was increase in plasma haemoglobin levels with increased storage time period.

TABLE 12:- COMPARISON OF HAEMOLYSIS AND PLASMA HB WITH OTHER STUDIES

Parameters	SAYEEDUL HASSAN ARIF ET AL[66]	M.I.CGYONGYOSSY-ISSA ET AL[67]	**PRESENT STUDY**
Pre Haemolysis (%) (WB)	0.081	0.04	0.046
Post Haemolysis (%) (LPC)	0.297	0.07	0.078
Pre Plasma Hb (mg/dL) (WB)	23.8	NA	12.23
Post Plasma Hb (mg/dL) (LPC)	149.9	NA	42.23

Serum Potassium levels: -

In our study at 0,14th and 28th day of collection the average potassium level was 3.74 mmol/l, 15.39 mmol/l and 20.69 mmol/l. whereas in local camps it was 4.22 mmol/l, 18.24 mmol/l and 23.19 mmol/l also in distant camp it was 4.37 mmol/l, 25.09 mmol/l and 31.93 mmol/l. similar results were shown by SayeedulHasan et al[66]studies in which on day 0 potassium value was 3.59 mmol/l that rose to 40.3 mmol/l up to day 28th. Also, Samuel Antwi- Baffour et al[70]study shows increase in potassium levels i.e on day 0 it was 3.54 mmol/l that rose to 15.36 mmol/l on day 28th. The results of the study agree with a similar study by Adias et al., which also recorded an increase in potassium concentration and decrease in sodium concentration during storage of blood.[71]

TABLE 13:- COMPARISON OF POTASSIUM WITH OTHER STUDIES

Parameters	SAYEEDUL HASSAN ARIF ET AL[66]	SAMUEL ANTWI-BAFFOUR ET AL[70]	**PRESENT STUDY**
Pre potassium (mmol/L) (WB)	3.59	3.54	3.74
Post potassium (mmol/L) (LPC)	40.3	15.6	20.69

Blood culture levels: -

Our study shows that no growth had occurred in indoor collections or distant or local camps after two weeks of incubation. In contrast to ShivaliSehgalet al[72]studies in which numerous known and unidentified pathogens have the ability to infect blood and blood components were found. The most frequently found microorganisms in donated blood are gram positive skin commensals, such as Bacillus cereus, Coagulase negative Staphylococci, Streptococcus sp., Staphylococcus aureus, Propionibacterium acnes, and gram-negative bacteria, such as Klebsiella spp., Serratia spp., Escherichia coli, Acinetobacter, Enterobacter spp. and Yersinia enterocolitica as in Brecher ME et al study.[73]

TABLE 14:- COMPARISON OF BLOOD CULTURE WITH ANOTHER STUDY

Parameters	ShivaliSehgal et al[72]	**PRESENT STUDY**
Blood culture (WB)	Growth present	No growth present
Blood culture (LPC)	Growth present	No growth present

Adverse donor events: -

This study shows that on days 0, 14, or 28, no adverse donor event from any blood unit happened during indoor collection. One patient had hypotension after receiving blood from the nearby camp collection, but another patient experienced (rash, fever) FNHTR after receiving blood from the distant camp collection. This could be due to the longer time

needed for component preparation and storage these results were comparable with Sandeepsahu et al study[74],which shows some transfusion related adverse events like FNHTR, Allergic reaction, TRALI and AHTR.

When compared to other Acute transfusion responses are responsible for the majority of significant adverse reactions and occurrences. A better prognosis can be achieved by being aware of the numerous clinical signs of acute transfusion responses and being able to assess significant reactions in a timely manner.

TABLE 15:- COMPARISON OF ADVERSE DONOR EVENTS WITH ANOTHER STUDY

Parameters	SANDEEPSAHU ET AL[74]	**PRESENT STUDY**
Adverse donor events	FNHTR, TRALI, AHTR, Allergic reaction	FNHTR, Hypotension

TABLE 16:- COMPARISON OF PRESENT STUDY WITH DIFFERENT STUDIES

PARAMETERS	SONYA HARISANTI ET AL[58]	JD SMITH ET AL[63]	EILAT SHINAR ET AL[64]	MUHAMMAD SARDAR ET AL[65]	SAYEEDUL HASSAN ARIF ET AL[66]	M.I.C GYONGYOSSY-ISSA ET AL[67]	SAMUEL ANTWI-BAFFOUR ET AL[70]	SHIVALI SEHGAL ET AL[72]	SANDEEP SAHU ET AL[74]	**PRESENT STUDY**
PRE HB(g/dl)	55.38	NA	NA	NA	NA	NA	NA	NA	NA	14.65
POST HB(g/dl)	59.72	NA	NA	NA	NA	NA	NA	NA	NA	19.89
PRE HCT (%)	69.4	NA	NA	NA	NA	NA	NA	NA	NA	45.76
POST HCT (%)	74	NA	NA	NA	NA	NA	NA	NA	NA	63.63
PRE LEUCOCYTE (10^3/µL)	NA	2.61	2.52	NA	NA	NA	NA	NA	NA	6.43
POST LEUCOCYTE (10^3/µL)	NA	0.62	0.69	NA	NA	NA	NA	NA	NA	0.01
PRE RBC(10^6/Ul)	NA	NA	NA	3.24	NA	NA	NA	NA	NA	4.94
POST RBC (10^6/Ul)	NA	NA	NA	2.62	NA	NA	NA	NA	NA	2.45
PRE PLASMA HB (mg/dl)	NA	NA	NA	NA	23.8	NA	NA	NA	NA	12.23
POST PLASMA HB (mg/dl)	NA	NA	NA	NA	149.9	NA	NA	NA	NA	42.23
PRE HAEMOLYSIS (%)	NA	NA	NA	NA	0.081	0.04	NA	NA	NA	0.046
POST HAEMOLYSIS (%)	NA	NA	NA	NA	0.297	0.07	NA	NA	NA	0.078
PRE POTASSIUM (mmol/l)	NA	NA	NA	NA	3.59	NA	3.54	NA	NA	3.74
POST POTASSIUM (mmol/l)	NA	NA	NA	NA	40.3	NA	15.36	NA	NA	20.69
BLOOD CULTURE	NA	NA	NA	NA	NA	NA	NA	GROWTH PRESENT	NA	NO GROWTH
ADVERSE DONOR EVENT	NA	NA	NA	NA	NA	NA	NA	NA	FNHTR, TRALI	FNHTR, HYPOTENSION

SUMMARY AND CONCLUSION

Our study showed that there was difference in the effect of storage duration on haemoglobin and haematocrit levels. Generally, this study aims to determine the average haemoglobin, haematocrit, RBC count, leucocyte count, haemolysis, plasma haemoglobin levels, serum potassium, blood culture and adverse reaction in indoor collection as well as in local and distant camps during day 0 to 14^{th} and 28^{th} day of storage period.

With the increase in the storage period there was rise in haemoglobin as well as haematocrit levels. Leucocyte count in pre filtration stage was higher as compare to post filtration. Leucocyte count was lower during post filtration that leads to lower log drop in WBC contents. RBC count also decreased during post filtration as well as with the increased storage time period of blood units. Percentage haemolysis was also increased with storage time period in this study however the extent of haemolysis did not exceed the permissible limit of 0.8% up to day 28^{th}. Also, there was rise in plasma haemoglobin level from day 0 to 28^{th} day of storage. Significant rise in potassium levels was seen with storage. However higher potassium values may be of little concern. Blood culture showed no growth after two weeks of incubation that helps in reducing the transfusion transmitted bacterial infections. Adverse donor event to a lesser extent seen in our study however observation or monitoring required throughout the transfusion episode for first 15 minutes for better prognosis. Indoor collection storage of blood showed less variation in these parameters as compare to local and distant camps collection that showed slightly higher variations in these parameters.

So, to conclude, these all parameters are very crucial for assessing the quality of stored blood unit and also to reduce adverse events related to blood transfusion as well as it shows that Whole blood filtration prior to component preparation seems to be useful alternative method for obtaining leukoreduced RBC and plasma with minimal whole blood loss.

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KEY TO MASTER CHART

HB	Haemoglobin
HCT	Haematocrit
K	potassium

Sr.No.	Group	GP	Blood unit	Pre_HB	Pre_RBC	Pre_HCT	Pre_LEUCOCYTE	Pre_plasma Hb	%HEMOLYSIS	HEMOLYSIS	Pre_s.k	14TH DAY Post_HB	14TH DAY POST RBC	14TH DAY Post HCT
1	INDOOR	1	137768	15.4	5.5	48.2	6.07	13.0	43.7	0.0437	3.8	18.5	2.1	59.4
2	INDOOR	1	137769	11.6	5.4	39.4	7.8	10.0	52.2	0.0522	3.4	12.7	3.7	40.0
3	INDOOR	1	137771	13.7	4.6	44.0	5.7	15.0	61.3	0.0613	3.6	14.9	2.4	50.7
4	INDOOR	1	137772	13.2	4.1	40.8	7.0	15.0	67.3	0.0673	3.4	14.6	2.6	49.7
5	INDOOR	1	137773	12.6	4.0	40.1	4.4	10.0	47.5	0.0475	3.1	14.0	2.4	48.3
6	INDOOR	1	137780	15.9	4.2	49.9	6.8	10.0	31.5	0.0315	3.9	16.5	3.6	55.0
7	INDOOR	1	137781	14.1	4.4	43.0	7.2	10.0	40.4	0.0404	3.3	14.5	2.6	46.8
8	INDOOR	1	137782	14.2	4.8	44.1	7.1	12.0	47.2	0.0472	3.8	17.4	3.0	55.3
9	INDOOR	1	135910	15.3	4.7	46.0	6.4	15.0	52.9	0.0529	4.0	17.4	2.2	60.2
10	INDOOR	1	135915	17.7	6.0	53.5	7.9	12.0	31.5	0.0315	3.8	18.8	3.8	62.1
11	INDOOR	1	135914	14.2	4.8	44.1	7.1	15.0	59.0	0.0590	2.5	15.1	1.8	51.1
12	INDOOR	1	135913	16.0	5.1	49.3	7.9	15.0	47.5	0.0475	4.6	19.7	3.4	63.4
13	INDOOR	1	137779	16.5	5.1	50.2	5.2	12.0	36.2	0.0362	3.8	20.5	3.2	62.6
14	INDOOR	1	137778	16.3	5.1	50.6	6.5	11.0	33.3	0.0333	3.6	20.0	3.2	63.9
15	INDOOR	1	137777	15.4	5.5	48.2	6.1	18.0	60.5	0.0605	3.9	18.9	3.6	59.8
16	INDOOR	1	137775	14.6	5.3	45.5	7.1	15.0	56.0	0.0560	3.4	19.3	3.1	62.5
17	INDOOR	1	137783	13.5	5.0	42.8	7.1	5.0	21.2	0.0212	3.1	14.3	3.2	44.3
18	INDOOR	1	137784	15.9	5.3	49.7	6.4	10.0	31.6	0.0316	4.6	18.9	2.7	61.6
19	INDOOR	1	135912	16.5	5.1	50.2	5.2	8.0	24.1	0.0241	3.3	22.2	4.4	72.6
20	INDOOR	1	134101	13.6	4.4	41.3	5.3	11.0	47.5	0.0475	4.5	17.4	2.2	55.3
21	INDOOR	1	123770	16.3	5.1	50.6	6.5	19.0	57.6	0.0576	3.4	20.6	1.5	64.8
22	INDOOR	1	123764	16.1	5.4	49.6	6.2	10.0	31.3	0.0313	4.6	21.9	2.3	70.6
23	INDOOR	1	123763	15.0	5.1	46.5	6.5	13.0	46.4	0.0464	4.5	18.5	4.3	59.8
24	INDOOR	1	123762	13.2	4.0	40.8	7.0	6.0	26.9	0.0269	3.3	17.9	1.6	56.1
25	INDOOR	1	123769	14.2	5.5	45.2	4.4	10.0	38.6	0.0386	3.7	16.7	2.1	53.9
26	INDOOR	1	112539	11.6	5.4	39.4	7.8	15.0	78.4	0.0784	4.5	15.7	2.9	48.7
27	INDOOR	1	112546	13.7	4.6	44.0	5.7	15.0	61.3	0.0613	4.2	15.3	3.7	46.0
28	INDOOR	1	112544	14.4	4.7	46.1	5.7	12.0	44.9	0.0449	3.5	17.7	2.0	53.5
29	INDOOR	1	119455	14.6	5.3	45.5	7.1	10.0	37.3	0.0373	4.1	17.9	4.0	56.1
30	INDOOR	1	121014	14.2	4.8	44.3	5.8	15.0	58.8	0.0588	3.5	16.7	4.0	53.9
31	LOCAL	2	118381	14.0	5.6	43.5	6.9	8.0	32.3	0.0323	3.9	16.6	3.1	52.5
32	LOCAL	2	118382	13.6	5.0	42.4	4.1	15.0	63.5	0.0635	4.6	17.0	2.4	51.0
33	LOCAL	2	118383	14.7	5.7	45.6	10.0	15.0	55.5	0.0555	3.3	10.7	2.9	32.4
34	LOCAL	2	118384	14.6	5.6	46.3	7.6	11.0	40.5	0.0405	4.5	19.0	3.0	62.2
35	LOCAL	2	115149	10.7	4.6	34.7	6.2	14.0	85.4	0.0854	3.4	16.5	3.2	51.7
36	LOCAL	2	115150	11.2	3.9	35.8	4.3	15.0	86.0	0.0860	4.6	17.6	1.1	56.3
37	LOCAL	2	115151	15.2	5.5	47.4	6.8	15.0	51.9	0.0519	4.5	19.8	3.9	61.4
38	LOCAL	2	126574	11.1	3.6	33.6	7.3	12.0	71.8	0.0718	5.2	19.3	2.0	58.8
39	LOCAL	2	126575	10.8	3.7	33.3	7.3	10.0	61.8	0.0618	4.7	19.1	2.6	60.0
40	LOCAL	2	112536	15.5	5.1	49.0	9.1	10.0	32.9	0.0329	4.9	18.0	4.3	56.3
41	LOCAL	2	112537	14.8	6.4	50.1	6.9	18.0	60.7	0.0607	3.9	19.1	4.0	59.8
42	LOCAL	2	112548	13.6	4.4	41.3	5.3	15.0	64.7	0.0647	2.9	16.5	2.2	51.7
43	LOCAL	2	112549	14.4	4.7	46.1	5.7	15.0	56.1	0.0561	3.3	18.5	3.9	59.8
44	LOCAL	2	112550	14.6	5.3	45.5	7.1	11.0	41.1	0.0411	3.8	11.1	2.9	33.8
45	LOCAL	2	135033	15.0	5.1	46.5	6.5	13.0	46.4	0.0464	4.2	14.2	2.7	44.3

Sr.No.	DAYPost_LEUCOCYTE	14TH DAY Post_plasma Hb	14TH DAY Post_s.k	14TH DAY %HEMOLYSIS	14th DAY HEMOLYSIS	28TH DAY Post HB	28TH DAY Post RBC	28TH DAY Post_HCT
1	0.0	18.0	10.6	39.5	0.0395	21.2	2.1	64.6
2	0.0	15.0	12.0	70.9	0.0709	16.5	3.6	55.0
3	0.0	32.0	11.7	105.9	0.1059	20.6	2.1	64.8
4	0.0	28.0	9.2	96.5	0.0965	18.5	2.3	59.8
5	0.0	25.0	19.8	92.3	0.0923	17.9	2.0	56.1
6	0.0	31.0	14.2	84.5	0.0845	19.7	3.1	62.4
7	0.0	18.0	19.9	66.0	0.0660	17.9	2.4	56.1
8	0.0	28.0	6.5	71.9	0.0719	20.4	2.7	64.5
9	0.0	36.0	14.4	82.3	0.0823	21.9	1.9	70.6
10	0.0	40.0	15.5	80.6	0.0806	20.6	3.4	64.8
11	0.0	55.0	10.3	178.1	0.1781	18.5	1.5	59.8
12	0.0	18.0	14.4	33.4	0.0334	21.2	3.1	64.6
13	0.0	25.0	12.8	45.6	0.0456	21.9	2.9	70.6
14	0.0	15.0	13.2	27.1	0.0271	20.6	2.7	64.8
15	0.0	18.0	15.6	38.3	0.0383	19.9	3.1	61.5
16	0.0	42.0	17.3	81.6	0.0816	21.9	2.7	70.6
17	0.0	35.0	13.7	136.3	0.1363	16.0	2.7	49.3
18	0.0	38.0	19.8	77.2	0.0772	19.7	2.2	63.4
19	0.0	45.0	10.4	55.5	0.0555	22.4	3.8	72.8
20	0.0	20.0	14.3	51.4	0.0514	19.7	1.6	63.4
21	0.0	26.0	20.6	44.4	0.0444	21.0	1.0	70.3
22	0.0	35.0	19.0	47.0	0.0470	22.4	1.9	72.8
23	0.0	40.0	17.5	86.9	0.0869	22.2	2.9	72.6
24	0.0	40.0	19.0	98.1	0.0981	21.0	1.2	70.3
25	0.0	15.0	20.0	41.4	0.0414	17.4	1.8	60.2
26	0.0	24.0	16.3	78.4	0.0784	18.9	2.4	61.6
27	0.0	28.0	18.9	98.8	0.0988	17.4	3.1	55.3
28	0.0	10.0	19.7	26.3	0.0263	20.5	1.3	62.6
29	0.0	15.0	14.3	36.8	0.0368	20.0	3.1	63.9
30	0.0	16.0	20.6	44.2	0.0442	18.9	3.1	59.8
31	0.0	15.0	19.0	42.9	0.0429	17.4	2.8	60.2
32	0.0	55.0	17.5	158.5	0.1585	18.9	2.0	61.6
33	0.1	45.0	19.0	284.3	0.2843	15.3	2.3	46.0
34	0.1	15.0	20.0	29.8	0.0298	19.3	2.5	61.4
35	0.0	20.0	16.3	58.5	0.0585	14.8	2.8	50.1
36	0.1	18.0	18.9	44.7	0.0447	12.7	1.0	41.1
37	0.0	38.0	19.7	74.1	0.0741	17.3	3.4	55.0
38	0.1	46.0	19.8	98.2	0.0982	21.9	1.7	70.6
39	0.0	25.0	18.1	52.4	0.0524	16.0	2.3	49.3
40	0.0	10.0	17.6	24.3	0.0243	19.7	3.6	63.4
41	0.0	40.0	13.0	84.2	0.0842	22.4	3.1	72.8
42	0.0	15.0	15.8	43.9	0.0439	19.7	1.8	63.4
43	0.0	20.0	20.2	43.5	0.0435	21.0	3.4	70.3
44	0.0	25.0	19.8	149.1	0.1491	22.4	2.7	72.8
45	0.0	46.0	17.4	180.4	0.1804	22.2	2.2	72.6

Sr.No.	28TH DAY Post_LEUCOCYTE	28TH DAY Post_plasma Hb	28TH DAY Post_s.k	28TH DAY %HEMOLYSIS	28TH DAY HEMOLYSIS	Blood culture	Adverse reaction
1	0.0	40.0	13.4	66.8	0.0668	No growth	NO
2	0.0	28.0	18.0	76.4	0.0764	No growth	NO
3	0.0	45.0	15.7	76.9	0.0769	No growth	NO
4	0.0	40.0	13.1	86.9	0.0869	No growth	NO
5	0.0	40.0	24.9	98.1	0.0981	No growth	NO
6	0.0	55.0	19.8	105.0	0.1050	No growth	NO
7	0.0	35.0	23.7	85.8	0.0858	No growth	NO
8	0.0	46.0	16.7	80.0	0.0800	No growth	NO
9	0.0	45.0	16.4	60.4	0.0604	No growth	NO
10	0.0	52.0	20.0	88.9	0.0889	No growth	NO
11	0.0	61.0	14.2	132.6	0.1326	No growth	NO
12	0.0	35.0	20.0	58.4	0.0584	No growth	NO
13	0.0	40.0	20.4	53.7	0.0537	No growth	NO
14	0.0	38.0	22.9	64.9	0.0649	No growth	NO
15	0.0	34.0	18.7	65.8	0.0658	No growth	NO
16	0.0	50.0	17.5	67.1	0.0671	No growth	NO
17	0.0	45.0	20.7	142.6	0.1426	No growth	NO
18	0.0	45.0	28.2	83.6	0.0836	No growth	NO
19	0.0	56.0	19.9	68.0	0.0680	No growth	NO
20	0.0	35.0	18.9	65.0	0.0650	No growth	NO
21	0.0	45.0	24.7	63.6	0.0636	No growth	NO
22	0.0	45.0	23.7	54.6	0.0546	No growth	NO
23	0.0	50.0	23.7	61.7	0.0617	No growth	NO
24	0.0	50.0	22.5	70.7	0.0707	No growth	NO
25	0.0	30.0	24.9	68.6	0.0686	No growth	NO
26	0.0	45.0	18.8	91.4	0.0914	No growth	NO
27	0.0	44.0	24.1	113.0	0.1130	No growth	NO
28	0.0	28.0	24.9	51.1	0.0511	No growth	NO
29	0.0	30.0	26.7	54.2	0.0542	No growth	NO
30	0.0	35.0	23.7	74.4	0.0744	No growth	NO
31	0.0	65.0	28.2	148.7	0.1487	No growth	NO
32	0.0	64.0	19.8	130.0	0.1300	No growth	NO
33	0.0	55.0	23.7	194.1	0.1941	No growth	NO
34	0.0	25.0	21.1	50.0	0.0500	No growth	NO
35	0.1	25.0	22.9	84.3	0.0843	No growth	NO
36	0.0	30.0	20.4	139.1	0.1391	No growth	NO
37	0.0	46.0	23.7	119.7	0.1197	No growth	NO
38	0.0	54.0	23.7	72.5	0.0725	No growth	NO
39	0.0	32.0	26.7	101.4	0.1014	No growth	NO
40	0.0	16.0	23.7	29.7	0.0297	No growth	NO
41	0.0	55.0	18.0	66.8	0.0668	No growth	NO
42	0.0	35.0	24.1	65.0	0.0650	No growth	NO
43	0.0	40.0	29.7	56.6	0.0566	No growth	hypotension
44	0.0	35.0	24.9	42.5	0.0425	No growth	NO
45	0.0	55.0	23.8	67.9	0.0679	No growth	NO

Sr.No.	Group	GP	Blood unit	Pre_HB	Pre_RBC	Pre_HCT	Pre_LEUCOCYTE	Pre_plasma Hb	%HEMOLYSIS	HEMOLYSIS	Pre_s.k	14TH DAY Post_HB	14TH DAY POST RBC	14TH DAY Post HCT
46	LOCAL	2	135035	13.2	4.0	40.8	7.0	8.0	35.9	0.0359	3.9	13.8	2.9	43.0
47	LOCAL	2	135016	15.2	5.5	47.4	6.2	5.0	17.3	0.0173	4.2	15.5	3.8	48.8
48	LOCAL	2	135004	15.9	5.3	49.7	6.4	10.0	31.6	0.0316	4.9	19.0	3.9	58.4
49	LOCAL	2	134995	13.8	4.9	33.8	4.4	12.0	57.6	0.0576	3.9	19.5	3.1	61.3
50	LOCAL	2	135011	14.2	6.2	44.3	6.6	15.0	58.8	0.0588	5.2	19.1	5.2	59.8
51	LOCAL	2	131181	15.5	5.1	49.0	9.1	16.0	52.6	0.0526	4.9	18.3	4.1	57.5
52	LOCAL	2	131182	12.3	4.1	38.7	5.8	15.0	74.8	0.0748	3.3	18.0	3.2	56.3
53	LOCAL	2	126152	11.2	4.1	33.8	4.2	15.0	88.7	0.0887	4.7	16.5	3.2	55.0
54	LOCAL	2	128715	14.4	4.7	46.1	4.0	14.0	52.4	0.0524	4.9	14.5	2.1	46.8
55	LOCAL	2	134055	13.7	4.5	40.0	4.0	15.0	65.7	0.0657	5.5	17.4	2.6	55.3
56	LOCAL	2	135874	14.6	5.3	45.5	7.1	14.0	52.3	0.0523	3.3	17.4	2.9	60.2
57	LOCAL	2	135870	15.4	5.5	48.2	6.1	15.0	50.5	0.0505	4.0	18.8	4.1	62.1
58	LOCAL	2	137710	14.2	4.8	44.3	5.8	15.0	58.8	0.0588	4.2	15.1	4.0	51.1
59	LOCAL	2	137708	14.5	5.3	46.3	7.5	10.0	37.0	0.0370	3.9	19.7	2.5	63.4
60	LOCAL	2	137645	13.6	4.4	41.3	5.3	10.0	43.2	0.0432	4.1	19.7	2.1	63.4
61	DISTANT	3	130181	15.0	5.1	46.5	6.5	16.0	57.1	0.0571	4.1	18.3	3.8	57.5
62	DISTANT	3	130182	13.2	4.0	40.8	7.0	12.0	53.8	0.0538	4.6	18.0	2.0	56.3
63	DISTANT	3	134118	14.2	5.5	45.2	4.4	10.0	38.6	0.0386	3.6	19.7	2.8	61.2
64	DISTANT	3	134117	11.6	5.4	39.4	7.8	12.0	62.7	0.0627	4.0	20.5	3.7	62.7
65	DISTANT	3	134116	13.7	4.6	44.0	5.7	12.0	49.1	0.0491	4.0	20.2	3.6	63.4
66	DISTANT	3	134115	14.4	4.7	46.1	5.7	18.0	67.4	0.0674	3.7	20.6	2.9	63.2
67	DISTANT	3	134114	14.6	5.3	45.5	7.1	12.0	44.8	0.0448	4.7	16.4	4.4	51.6
68	DISTANT	3	134113	14.2	4.8	44.3	5.8	15.0	58.8	0.0588	4.3	19.5	3.2	61.7
69	DISTANT	3	134112	14.0	5.6	43.5	6.9	15.0	60.5	0.0605	4.7	18.3	3.9	57.0
70	DISTANT	3	134107	13.6	5.0	42.4	4.1	12.0	50.8	0.0508	3.5	20.5	3.1	61.8
71	DISTANT	3	134108	14.7	5.7	45.6	10.0	12.0	44.4	0.0444	3.8	20.3	4.0	63.2
72	DISTANT	3	134109	14.6	5.6	46.3	7.6	10.0	36.8	0.0368	3.6	22.4	3.8	66.6
73	DISTANT	3	134110	10.7	4.6	34.7	6.2	10.0	61.0	0.0610	4.0	19.1	2.8	60.5
74	DISTANT	3	135917	11.2	3.9	35.8	4.3	12.0	68.8	0.0688	4.0	18.7	1.9	62.1
75	DISTANT	3	135918	15.2	5.5	47.4	6.8	10.0	34.6	0.0346	3.8	19.4	3.9	61.8
76	DISTANT	3	130183	11.1	3.6	33.6	7.3	15.0	89.7	0.0897	4.1	16.6	2.9	51.8
77	DISTANT	3	130184	10.8	3.7	33.3	7.3	15.0	92.6	0.0926	5.0	14.2	1.9	44.3
78	DISTANT	3	130185	15.5	5.1	49.0	9.1	20.0	65.8	0.0658	5.0	19.0	4.0	57.0
79	DISTANT	3	130186	14.8	6.4	50.1	6.9	14.0	47.2	0.0472	5.2	18.2	5.0	56.0
80	DISTANT	3	134056	13.6	4.4	41.3	5.3	12.0	51.8	0.0518	4.5	18.5	2.0	59.4
81	DISTANT	3	134057	14.4	4.7	46.1	5.7	15.0	56.1	0.0561	5.0	12.7	3.0	40.0
82	DISTANT	3	134058	14.6	5.3	45.5	7.1	25.0	93.3	0.0933	4.5	14.9	3.0	50.7
83	DISTANT	3	134059	15.0	5.1	46.5	6.5	15.0	53.5	0.0535	3.4	14.6	3.1	49.7
84	DISTANT	3	134060	15.4	5.5	48.2	6.1	15.0	50.5	0.0505	4.0	14.0	3.5	48.3
85	DISTANT	3	126153	11.6	5.4	39.4	7.8	10.0	52.2	0.0522	2.1	16.5	3.4	55.0
86	DISTANT	3	126154	13.7	4.6	44.0	5.7	20.0	81.8	0.0818	5.5	14.5	2.8	46.8
87	DISTANT	3	126155	13.2	4.1	40.8	7.0	10.0	44.8	0.0448	6.0	17.4	2.6	55.3
88	DISTANT	3	126187	12.6	4.0	40.1	4.4	15.0	71.3	0.0713	6.7	17.4	2.6	60.2
89	DISTANT	3	118385	15.9	4.2	49.9	6.8	15.0	47.3	0.0473	4.7	18.8	2.6	62.1
90	DISTANT	3	118386	13.6	5.0	42.4	4.1	12.0	50.8	0.0508	5.6	15.1	3.2	51.1

Sr.No.	DAYPost_LEUCOCYTE	14TH DAY Post_plasma Hb	14TH DAY Post_s.k	14TH DAY %HEMOLYSIS	14th DAY HEMOLYSIS	28TH DAY Post HB	28TH DAY Post RBC	28TH DAY Post_HCT
46	0.0	30.0	17.2	123.9	0.1239	21.0	2.4	70.3
47	0.0	68.0	18.3	224.6	0.2246	17.4	3.4	60.2
48	0.0	65.0	20.5	142.3	0.1423	18.9	3.3	61.6
49	0.0	58.0	16.6	115.1	0.1151	17.4	2.9	55.3
50	0.0	35.0	22.4	73.7	0.0737	20.5	4.7	62.6
51	0.0	55.0	19.1	127.7	0.1277	18.3	3.9	57.0
52	0.0	50.0	18.7	121.4	0.1214	20.5	3.0	61.8
53	0.0	36.0	19.4	98.2	0.0982	20.3	3.0	63.2
54	0.0	28.0	16.6	102.7	0.1027	22.4	1.9	66.6
55	0.0	40.0	16.8	102.8	0.1028	19.1	2.2	60.5
56	0.0	25.0	19.0	57.2	0.0572	18.7	2.4	62.1
57	0.0	50.0	18.9	100.8	0.1008	19.4	3.9	61.8
58	0.0	76.0	18.5	246.1	0.2461	16.6	3.7	51.8
59	0.0	25.0	15.2	46.4	0.0464	21.2	2.1	64.6
60	0.0	80.0	18.1	148.6	0.1486	16.5	1.9	55.0
61	0.0	45.0	19.0	104.5	0.1045	20.6	3.7	64.8
62	0.0	45.0	16.7	109.3	0.1093	18.5	1.9	59.8
63	0.0	46.0	21.0	90.6	0.0906	17.9	2.6	56.1
64	0.0	68.0	29.3	123.7	0.1237	19.7	3.4	62.4
65	0.0	55.0	26.8	99.7	0.0997	17.9	3.1	56.1
66	0.0	80.0	32.1	142.9	0.1429	20.4	2.7	64.5
67	0.0	45.0	18.8	132.8	0.1328	21.9	4.0	70.6
68	0.0	60.0	27.6	117.8	0.1178	20.6	2.9	64.8
69	0.0	75.0	24.0	176.2	0.1762	18.5	3.8	59.8
70	0.0	55.0	26.7	102.5	0.1025	21.2	2.9	64.6
71	0.0	55.0	22.1	99.7	0.0997	21.9	3.8	70.6
72	0.0	60.0	18.3	89.5	0.0895	20.6	3.8	64.8
73	0.0	48.0	20.0	99.3	0.0993	19.9	2.6	61.5
74	0.0	65.0	24.6	131.7	0.1317	21.9	1.6	70.6
75	0.0	50.0	21.0	98.5	0.0985	16.0	3.5	49.3
76	0.0	25.0	19.0	72.6	0.0726	19.7	2.5	63.4
77	0.0	65.0	23.7	255.0	0.2550	22.4	1.6	72.8
78	0.0	86.0	19.0	194.6	0.1946	19.7	3.3	63.4
79	0.0	55.0	24.9	133.0	0.1330	21.0	4.6	70.3
80	0.0	60.0	29.4	131.7	0.1317	22.4	1.8	72.8
81	0.0	50.0	22.5	236.2	0.2362	18.3	2.6	57.5
82	0.0	20.0	24.9	66.2	0.0662	18.0	2.6	56.3
83	0.0	40.0	30.5	137.8	0.1378	19.7	2.8	61.2
84	0.0	45.0	26.7	166.2	0.1662	20.5	3.0	62.7
85	0.0	56.0	23.7	152.7	0.1527	20.2	3.0	63.4
86	0.0	42.0	28.2	154.1	0.1541	20.6	2.7	63.2
87	0.0	64.0	23.8	164.4	0.1644	16.4	2.3	51.6
88	0.0	60.0	41.1	137.2	0.1372	19.5	2.3	61.7
89	0.0	60.0	38.2	121.0	0.1210	18.3	2.4	57.0
90	0.0	50.0	29.1	161.9	0.1619	20.5	3.0	61.8

Sr.No.	28TH DAY Post_LEUCOCYTE	28TH DAY Post_plasma Hb	28TH DAY Post_s.k	28TH DAY %HEMOLYSIS	28TH DAY HEMOLYSIS	Blood culture	Adverse reaction
46	0.0	40.0	21.0	56.6	0.0566	No growth	NO
47	0.0	85.0	26.7	194.4	0.1944	No growth	NO
48	0.0	72.0	25.8	146.3	0.1463	No growth	NO
49	0.0	70.0	20.0	179.8	0.1798	No growth	NO
50	0.0	45.0	20.4	82.1	0.0821	No growth	NO
51	0.0	64.0	22.7	150.4	0.1504	No growth	NO
52	0.0	61.0	21.1	113.7	0.1137	No growth	NO
53	0.0	45.0	22.9	81.6	0.0816	No growth	NO
54	0.0	36.0	20.4	53.7	0.0537	No growth	NO
55	0.0	55.0	23.7	113.7	0.1137	No growth	NO
56	0.0	40.0	23.7	81.1	0.0811	No growth	NO
57	0.0	65.0	26.7	128.0	0.1280	No growth	NO
58	0.0	90.0	23.7	261.3	0.2613	No growth	NO
59	0.0	35.0	18.0	58.4	0.0584	No growth	NO
60	0.0	90.0	24.8	245.5	0.2455	No growth	NO
61	0.0	55.0	27.1	94.0	0.0940	No growth	NO
62	0.0	55.0	25.0	119.5	0.1195	No growth	NO
63	0.0	111.0	29.0	272.2	0.2722	No growth	NO
64	0.0	75.0	38.4	143.1	0.1431	No growth	NO
65	0.0	63.0	33.4	154.5	0.1545	No growth	NO
66	0.0	95.0	46.5	165.3	0.1653	No growth	NO
67	0.0	110.0	24.1	147.7	0.1477	No growth	NO
68	0.0	133.0	31.1	227.3	0.2273	No growth	NO
69	0.0	146.0	29.7	317.3	0.3173	No growth	NO
70	0.0	64.0	31.2	106.9	0.1069	No growth	NO
71	0.0	85.0	29.3	114.1	0.1141	No growth	fever
72	0.0	73.0	22.5	124.7	0.1247	No growth	NO
73	0.0	60.0	24.9	116.1	0.1161	No growth	NO
74	0.0	75.0	30.5	100.7	0.1007	No growth	NO
75	0.0	112.0	26.7	354.9	0.3549	No growth	NO
76	0.0	40.0	23.7	74.3	0.0743	No growth	NO
77	0.0	85.0	28.2	103.2	0.1032	No growth	NO
78	0.0	100.0	23.8	185.8	0.1858	No growth	NO
79	0.0	55.0	41.1	77.8	0.0778	No growth	NO
80	0.0	80.0	38.2	97.1	0.0971	No growth	NO
81	0.0	105.0	38.7	243.9	0.2439	No growth	NO
82	0.0	40.0	46.5	97.1	0.0971	No growth	rashes
83	0.0	55.0	33.4	108.3	0.1083	No growth	NO
84	0.0	180.0	29.0	327.5	0.3275	No growth	NO
85	0.0	65.0	27.1	117.8	0.1178	No growth	NO
86	0.0	60.0	41.9	107.2	0.1072	No growth	NO
87	0.0	86.0	38.4	253.8	0.2538	No growth	NO
88	0.0	155.0	36.3	304.4	0.3044	No growth	NO
89	0.0	140.0	29.1	329.0	0.3290	No growth	NO
90	0.0	68.0	33.2	126.7	0.1267	No growth	NO

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Receipt No. 3521
Date 18/3/24
Principal's Office

The Principal
Dayanand Medical College & Hospital
Ludhiana

Subject:: Approval of Plan of Thesis.

This is to inform you that the Faculty of Medical Sciences in its meeting held on 25.09.2023 has approved the plan of thesis of **Harleen Kaur**, a student of your college for **MD Immuno Haematology & Blood Transfusion** along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis
Effect of delayed leukoreduction on rbc after prolong storage by use of inline filter .

Supervisor
Dr. Rajesh Kumar
Professor & Head
Dept. of I.H.B.T.

Co-Supervisor-
Dr. Bhavna Malhotra
Professor
Dept. Of Pathology

The student may submit the thesis in triplicate alongwith the plan in triplicate also, to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She may be directed to attach a photocopy of this letter with the forwarding letter while submitting thesis in the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic, if any.

Controller of Examinations

Note :
1. *If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.*

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Controller of Examinations

Note :

1. *If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.*

EFFECT OF DELAYED LEUKOREDUCTION ON RBC AFTER PROLONG STORAGE BY USE OF INLINE FILTER

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MD

IN

IMMUNO HEMATOLOGY AND BLOOD TRANSFUSION
OF THE

BABA FARID UNIVERSITY OF HEALTH SCIENCES
FARIDKOT

AUGUST, 2022

DR. HARLEEN KAUR

DEPARTMENT OF IMMUNO HEMATOLOGY AND BLOOD TRANSFUSION
DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA

ABSTRACT OF PLAN OF THESIS

Title	EFFECT OF DELAYED LEUKOREDUCTION ON RBC AFTER PROLONG STORAGE BY USE OF INLINE FILTER.
For the degree of	MD I.H.B.T
Name of the candidate	DR. HARLEEN KAUR
Supervisor	DR. RAJESH KUMAR PROFESSOR, DEPARTMENT OF I.H.B.T, DAYANAND MEDICAL COLLEGE AND HOSPITAL
Co-supervisor	DR. AMARJIT KAUR, PROFESSOR & HEAD, DEPARTMENT OF I.H.B.T, DAYANAND MEDICAL COLLEGE AND HOSPITAL DR. BHAVNA MALHOTRA, PROFESSOR, DEPARTMENT OF PATHOLOGY, DMCH, LUDHIANA
Institution	DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA
University	BABA FARID UNIVERSITY OF HEALTH SCIENCES, FARIDKOT

Introduction: Blood transfusion is an indispensable part of patient management, like all therapeutic procedures it has also some adverse effects. However, few complications of blood transfusion arise due to presence of leukocytes in donor's blood. Leukoreduction is the removal of leukocytes from blood components. Leukoreduction of components is a widely practiced and established strategy for reducing the incidence of transfusion associated adverse event.

Aim Of Study: To evaluate the efficiency and quality of pre storage leukocyte reduction of whole blood following delayed storage at ambient temperature.

Materials And Methods: Blood will be collected from random voluntary blood donors into quadruple bag with inline filter for whole blood, The collected bags will be divided into 3 groups of 30 units each.

Group A Will contain the indoor collected blood units in quadruple bag with inline filter for whole blood which will be kept for 12-18 hours at 4-6°C before filtration and separation, the leukocyte depleted red blood cell concentrate (RBCC) prepared from these units will be processed and stored at 4-6° C for 28 days.

Group B and Group C will contain the collected blood units from local and distant camps and kept for 12-18 hours at 4-6°C before filtration and processing, these leukocyte depleted RBCC will be stored at 4-6° C for 28 days.

Aseptic samples will be assessed for complete blood count (Hb, RBC count, haematocrit, leukocyte), plasma Hb, RBC haemolysis, serum electrolytes (Na-K+) and blood culture at pre and post filtration stages as well as during storage on fortnight basis thereafter

Significance: Whole blood filtration prior to component preparation seems to be useful alternative method for obtaining leukoreduced RBC and plasma with minimal whole blood loss.

Keywords: Leukocytes, Leukoreduction, Febrile non haemolytic transfusion reaction, Red blood cell concentrate.

Harleen
(Signature)
Candidate

(Signature)
Supervisor

Amarjit
(Signature)
Co-supervisor

Bhavna
(Signature)
Co- supervisor

(Signature)
Principal

CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"EFFECT OF DELAYED LEUKOREDUCTION ON RBC AFTER PROLONG STORAGE BY USE OF INLINE FILTER"** exist in the Department of Immuno hematology and Blood Transfusion at DMC&H, Ludhiana and will be provided to be candidate of will be provided to the candidate. We will see that the data being included in the thesis are genuine and is collected by the candidate herself under our supervision and guidance.

Supervisor

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Professor
Department of I.H.B.T
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Co-Supervisors

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Head of the Department

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Date: 30/9/22

APPROVAL PROFORMA
BY RESEARCH & ETHICAL COMMITTEE
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Name of candidate	DR. HARLEEN KAUR
Department	I.H.B.T
Topic of Thesis	EFFECT OF DELAYED LEUKOREDUCTION ON RBC AFTER PROLONG STORAGE BY USE OF INLINE FILTER.
Likely date of appearing in PG examination	May/June, 2024
Date of enrollment	2nd May, 2022
Name of Head of Department	DR. AMARJIT KAUR
1. Supervisor **2. Co-Supervisor**	DR. RAJESH KUMAR DR. AMARJIT KAUR DR. BHAVNA MALHOTRA
Signature of Members of Research Committee with Stamp	**Signature of Members of Ethics Committee with Stamp**
Dr. HarpreetKaur (Professor & Head, Pathology)	Mr. R K Sharma (Legal Expert)
Dr. Jaspal Singh (Professor & Head, Surgery)	Dr. RamitMahajan (Associate Professor, Gastroenterology)
Dr. Dinesh Gupta (Professor & Head, Medicine)	Dr. Anu Sharma (Professor, Anatomy)
Dr. SandeepKaushal (Dean Academics & Professor & Head, Pharmacology)	Dr. SandeepKaushal (Dean Academics & Professor & Head, Pharmacology)
Dr. Gurpreet S Wander (Vice Principal)	Dr. Gurpreet S Wander (Vice Principal & Professor & Head, Cardiology)
	Dr. Gagandeep Singh (Secretary, Ethics Committee Professor & Head, Neurology)

Approved: Yes / ~~No~~

Dr. SandeepPuri
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Research Committee

Chairperson
Research Committee

Approved: Yes / ~~No~~

Prof. ArvindMalhotra
Chairperson
Ethics Committee

Chairperson
Ethics Committee

BABA FARID UNIVERSITY OF HEALTH SCIENCES,

FARIDKOT

PROFORMA FOR REGISTRATION OF SUBJECT FOR DISSERTATION

1	NAME OF THE CANDIDATE AND ADDRESS	DR. HARLEEN KAUR H. NO 58, ARORA COLONY, KAKKON, HOSHIARPUR
2	NAME OF THE INSTITUTE	DAYANAND MEDICAL COLLEGE AND HOSPITAL, LUDHIANA
3	COURSE OF STUDY AND SUBJECT	MD IMMUNO HEMATOLOGY AND BLOOD TRANSFUSION
4	DATE OF ADMISSION OF COURSE	02/05/2022
5	TITLE OF THE TOPIC	EFFECT OF DELAYED LEUKOREDUCTION ON RBC AFTER PROLONG STORAGE BY USE OF INLINE FILTER.

6. BRIEF RESUME OF THE INTENDED WORK

6.1 NEED FOR THE STUDY

Blood transfusion is an indispensable part of patient management. However, like all therapeutic procedures it has its share of adverse effects. However few complications of blood transfusion arise due to presence of leukocytes in donor's blood. The advancement of procedures to reduce the content of donor leukocytes has contributed significantly in bringing down related adverse effects. The white blood cells present in blood components can be associated with adverse effects after transfusion. These include primary HLA alloimmunization with consequent refractoriness to platelet transfusion[1], febrile non haemolytic transfusion reactions and transmission of viruses such as cytomegalovirus and human T cell lympho trophic viruses. WBC reduction in blood components may be achieved by filtration with currently available leukocyte depletion filters. Recent studies indicate that third generation WBC reduction filters consistently achieve more than 3 $\log_{10}$ reduction.[2] It is an indispensable part of patient management. But few complications arise due to presence of leukocytes in donor blood. The advancement of procedures to reduce the content of donor leukocytes.

Leukoreduction is the removal of leukocytes from blood components. Each leukoreduced product had residual leukocyte counts of less than $5*10^6$ cells per unit. At present leukoreduction is performed using leukocyte reduction filters that trap leukocytes, while allowing the desired blood product to pass through . Thus the removal of leukocytes below a certain threshold $<5*10^6$ in a blood component certainly helps in prevention of alloimmunization and associated risks in these patients. Currently the best leukoreduction can be achieved with the help of 3rd and 4th generation leukofilters, both in laboratory and patient bedside and state of the art apheresis devices, But pre storage leukoreduction is better than bedside.[3]

There is evidence that WBC and platelet fragmentation is rapid during storage and that degradation products such as histamine, elastase, acid phosphatase and There serotonin accumulate if WBC and platelets are not removed before storage. Reports indicate that pre storage WBC depletion of red blood cell concentrates improves the storage characteristics of the units: less haemolysis, lower supernatant potassium, better post storage 2,3-DPG and ATP values.[4]

Recently in-line filtration has been introduced which allows early WBC depletion of RBCC in closed systems before storage. However integral RBCC filtration requires that blood bags and filter be centrifuged which may lead to reduced centrifuge capacity and require accurate cup packing. Whole blood filtration may well improve the RBCC storage parameters maintaining plasma coagulation factors.[5]

6.2 REVIEW OF LITERATURE

Blood transfusion is a fundamental therapy in numerous pathological conditions. Many clinical reports describe adverse transfusion's drawbacks due to red blood cells alteration during storage.[6] Thus the possibility for a blood bank to ameliorate the quality of the erythrocyte concentrates units is crucial to improve results and reduce transfusion reactions.[6] All leukocytes are produced from common pluripotent hematopoietic stem cell under the effect of various growth factors known as cytokines. Further leukocytes divided into two broad categories, granulocytes (neutrophils, basophils, eosinophils) and agranulocytes (lymphocytes, monocytes). Cytokines act at various stages and in coordination so as to induce leukocyte proliferation, maturation and function.[7]

Cytokines influence development and maintenance of inflammation and immunity. Also cytokines release from leukocytes depends on the type of cellular interactions. Biological effects of cytokines release include fever, chills, rigors and increased capillary permeability.[7]

Clinical studies identified a correlation between transfusion of red blood cells stored for more than 14 days and the increase of infectious events, extension of hospitalization time, prolonged mechanical ventilation, multiple organ failure and mortality. The origin of these negative outcomes are attributed to the morphological and biochemical modification that RBC's undergo during prolong storage e.g. the production of lactate leads to a decrease in Ph, depletion in adenosine triphosphate and 2,3 diphosphoglycerate. Storage at low temperature (2-6 degree Celsius) slows the rate of glycolysis , unfortunately it also decreases the activity of the Na+ K+ concentration in the conservation medium augments the risk of arrythmia in case of rapid transfusion through a central vein. Storage is also known to induce changes in RBC's morphology as well as membrane loss. In this respect, the exact correlation between those storage lesions

and the fate of the transfused cells is still unknown and calls the attention of the scientific community.[8]

A subject of major debate among clinical practitioners is the definition of the criteria for the selection of the transfusion units: the prevalent opinion, points to the storage time of the bags. While there are data indicating a correlation between RBC's storage time and morbidity/ mortality of the transfused patients, randomized controlled trials are in progress to verify this fact. Consequent to these consideration, reduction of the RBS's storage time would be a simple solution. The methods to prolong the quality of RBC's during storage are being searched: the removal of leukocytes, before storage is a promising one.[6]

The origin of the beneficial outcomes associated to the introduction of RBC's leukodepletion before storage, are expected to be due to the preservation of RBC's quality for a longer time. Because of the main difference between leuko reduced and leuko depleted RBC's is the significantly reduced numbers of leukocytes present in the bags of leukodepleted RBC's to progress in the optimization of RBC's storage , it is necessary to understand how the presence of leukocytes influence RBC's vitality a condition that at present is generally associated to the maintenance of both their proper shape and efficient metabolism.[6]

The removal of WBC from cellular blood components is associated with reduction of several transfusion- associated adverse reactions. These include febrile, nonhemolytic transfusion reactions, HLA alloimmunization with subsequent refractoriness to platelet transfusion, transfusion associated GVHD, immunosuppression and transmission of certain infectious leukotrophic agents.[9]

Febrile non-haemolytic transfusion reaction (FNHTR): Rise in temperature of 1 degree Celsius or more above the pre transfusion baseline, in the absence of any underlying febrile illness. It usually occurs

during or within few hours of transfusion and can be associated with chills, nausea, vomiting, tachycardia, hypertension. However asymptomatic rise in body temperature in a hypothermic patient to normal temperature is not FNHTR.[10]

Cytokines release is the primary event responsible for symptoms in FNHTR. There are two main mechanisms leading to cytokines release. The first is due to interaction between recipient antibodies and antigens on donor leukocytes. The antibodies are directed against antigens both HLA and WBC specific found on donor leukocytes.[11] Also the severity of relation is determined by the extent of cytokines release in response to antigen antibody interactions. The second mechanism is due to infusion of preformed cytokines and inflammatory mediators that accumulate in plasma based blood components especially platelet rich concentrate upon storage. These cytokines are released into the plasma from WBC's storage. The final step in both pathways is by increasing the levels of cytokines in the recipient like interleukin-1 beta, IL6 and tumour necrosis factor. Cytokines induce fever by upregulating thermostatic set points in the thermoregulatory centre of the hypothalamus.[12]

HLA Alloimmunization: It is delayed immune complication of blood transfusion. Also a major cause of the platelet refractory state. Exposure to HLA class 1 antigens are implicated in primary alloimmunization and they can be present in blood component in three forms : on intact cells, on degenerating cells and cell fragments, and in soluble form on plasma lipoproteins. Development of anti-HLA antibodies following alloimmunization is one of the major reason behind platelet refractoriness and failure in platelet increment following platelet transfusion especially in patients with hematologic malignancies.[13]

Transfusion associated graft versus host disease (TA-GVHD): It results from donor T lymphocytes reacting against proteins on host cells, proliferating and targeting host organ primarily the liver, skin, intestinal

tract. It is usually seen with whole blood or red cell transfusion but can be associated with fresh frozen plasma and platelet concentrates also, if there are viable lymphocytes in them.[12]

Leukocyte filter: A typical filter design consists of a fibre material that can be sterilized, upstream layers which prevent clotting, a plastic housing and inlet/outlet ports which prevent retention of air bubbles and possibility to have a tight seal between filter layers and housing to prevent channeling.[14] There are two types of filtration mechanism: surface/screen filtration and depth filtration. Depth filter has filter material in the form of compressed wool fibres arranged in an irregular fashion whereas screen filter have fibres arranged in multiple layers in a regular fashion.[15] In screen filtration separation of particles is restricted to filter surface while it is not the same in depth filtration. In depth filtration which is the most highly regarded mechanism for leukocyte reduction, the porous structure has wide distribution of pore sizes throughout the filter matrix, which allows retention of particles at any place inside the filter. Leukocyte capture in filters occur by mechanical sieving, direct adhesion and cell-cell interaction.

Timing of leuko filtration: It can be done either at the time of collection and processing, post processing (within the blood bank) or by the side of the patient (post storage). The advantages of pre storage over post storage leukoreduction are as follow:

- It eliminate the scope of inflammatory (interleukins-1, interleukins-6, tumour necrosis factor) cytokine accumulation due to leukocytes, during storage and hence is quite efficient in the prevention of febrile non haemolytic transfusion reactions.[16-18]
- It also minimizes the risk of HLA alloimmunization in multi transfused patients as it removes the intact leukocytes as against filtration at the bedside here leukocyte fragments after storage can

pass through filters and allo immunize the recipient against donor antigens.[19-20]

- Pre storage leukofiltration can also minimize the risk of leukotropic virus transmission as leukocytes disintegrate and release the intracellular organism after 72 hours of storage in blood components.[21-22]
- It is always easier to perform leukocyte quality control in the laboratory rather than by the patients bedside, hence during pre storage leukoreduction blood components can be thoroughly studied and evaluated for quality control and various factors affecting the process of leukofiltration modified accordingly.[23-24]

Contribution of each mechanism of filtration depends upon:- types of fibres, physical properties of blood cells, composition of blood components, filtration method[9], critical points in the process of filtration of red cell filters are leukocyte and platelet number and presence of plasma, temperature, age of the product, flow, priming and rinsing.[25]

Selective pre storage leukofiltration for patients on regular transfusion:- The packed red cell selective pre storage leukofiltration policy can be adopted for patients on regular transfusion therapy such as thalassemia major patients with skill full inventory management and active coordination between the transfusion therapy clinic and the blood bank. Platelets concentrates can be pooled and leukofiltered for a selected group of haemato-oncology patients in the blood centres prior to the storage. This strategy can achieve 3-4 log leukoreduction however it require dedicated technical manpower for stringent quality control and skill full inventory management along with active coordination with the treating units.[25]

6.3 AIMS AND OBJECTIVES OF THE STUDY

- To evaluate the efficiency and quality of pre storage leukocyte reduction of whole blood, following delayed storage at ambient temperature.

7. MATERIALS AND METHODS

Blood will be collected from random voluntary blood donors into quadruple bag with inline filter for whole blood, containing 63 ml of citrate phosphate dextrose (CPD) anticoagulant and saline adenine glucose mannitol (SAGM) as RBC preservative solution. The collected bags will be divided into 3 groups of 30 units each.

Group A will contain the indoor collected blood units in quadruple bag with inline filter for whole blood which will be kept for 12-18 hours at 4-6° C before filtration and separation, the leukocyte depleted red blood cell concentrate (RBCC) prepared from these units will be processed and stored at 4-6°C for 28 days.

Group B will contain the collected blood units from the local camp and these collected units kept for 12-18 hours at 4-6°C before filtration and separation and the leukocyte depleted red blood cell concentrate (RBCC) prepared from these units will be processed and stored at 4-6° C for 28 days.

Group C will contain the collected blood units from the distant camp and these collected units kept for 12-18 hours at 4-6° C before filtration and separation, the leukocyte depleted red blood cell concentrate (RBCC) prepared from these units will be processed and stored at 4-6° C for 28 days.

Aseptic samples will be assessed for complete blood count (Hb, RBC count, haematocrit, leukocyte), plasma Hb, RBC haemolysis, serum electrolytes (Na-K+) and blood culture at pre and post filtration stages as well as during storage on fortnight basis thereafter.

7.1 SOURCE OF DATA

- The study will be conducted in the Immuno hematology and Blood Transfusion department and pathology department of Dayanand medical college and hospital.

7.2 METHOD OF COLLECTION OF DATA

INCLUSION CRITERION

- 450±10% ml of blood collected in quadruple bag with inline filter for whole blood.

EXCLUSION CRITERION

- Over volume bag
- Reactive units
- Insufficient amount collected units

DURATION OF STUDY

One year, 1st January, 2023 to 31st December, 2023

OUTCOME

Whole blood filtration prior to component preparation seems to be useful alternative method for obtaining leukoreduced RBC and plasma with minimal whole blood loss and may also reduce adverse reactions in transfused patients.

STATISTICAL ANALYSIS

Data will be described in terms of range; mean ±standard deviation (± SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. To determine whether the data is normally distributed, a Kolmogorov-Smirnov test will be used. Comparison of

quantitative variables between the study groups will be done using ANOVA and Kruskal-Wallis Test with post hoc Tukey's for independent samples for parametric and non-parametric data respectively. For comparing categorical data, Chi square ($\chi 2$) test will be performed and fisher exact test will be used when the expected frequency is less than 5. A probability value (p value) less than 0.05 will be considered statistically significant. All statistical calculations will be done using (Statistical Package for the Social Science) SPSS 21version (SPSS Inc., Chicago, IL, USA) statistical program for Microsoft Windows.

7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.

No

7.4 HAS ETHICAL CLEARENCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?

Yes, Attached herewith.

8. LIST OF REFERENCES

1. Rapaille A, Moore G, Siquet J, Flament J, Sondag-Thull D. Prestorage leukocyte reduction with in-line filtration of whole blood: evaluation of red cells and plasma storage. Vox sanguinis. 1997;73:28-35.
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PROFORMA

BLOOD BAG UNIT NUMBER:-

BLOOD BAG LOT NUMBER:-

DATE OF COLLECTION:-

DATE OF EXPIRY:-

TIME OF COLLECTION OF BLOOD UNITS:-

TRANSPOTATION TIME:-

PRE STORAGE COMPLETE BLOOD COUNT:- Hb

RBC COUNT

HAEMATOCRIT

LEUKOCYTE

POST STORAGE COMPLETE BLOOD COUNT:- Hb

RBC COUNT

HAEMATOCRIT

LEUKOCYTE

PLASMA Hb

RBC HAEMOLYSIS

SERUM ELECTROLYTES: POTASSIUM

BLOOD CULTURE:-

ADVERSE TRANSFUSION REACTION:-

Signature of Candidate **Signature of Supervisor**

INFORMED PATIENT CONSENT

Date: _____

I……..D/o,W/o,S/o…… ……. ……am willing to participate in the study **"EFFECT OF DELAYED LEUKOREDUCTION ON RBC AFTER PROLONG STORAGE BY USE OF INLINE FILTER"** The procedure with its complications has been explained to me in local vernacular language. I have fully understood that no special tests and extra expenditure in any form, merely for the purpose of thesis is being carried out. I am free to opt out the study any time without assigning any reason.

……	……
Signature of the Witness	Signature of the patient
Name:	

Signature of Candidate **Signature of Supervisor**

मरीज अनुमति फार्म

तिथि _____

मैं _____ पत्नी/पुत्र/पुत्री _____

निवासी _____ इस शोधकार्य **"EFFECT OF DELAYED LEUKOREDUCTION ON RBC AFTER PROLONG STORAGE BY USE OF INLINE FILTER"** में भाग लेने के लिए सहमत हूँ। मैं इस शोधकार्य में भाग लेने के लिए अपनी स्वेच्छा से तैयार हूँ। मैं पुष्टि करता/करती हूँ की यह स्वीकृति देने के लिए मुझे कोई आर्थिक प्रलोभन नहीं दिया गया और न ही दबाव डाला गया है। मुझे इस शोधकार्य का उद्देश्य स्पष्ट कर दिया गया है और मुझे यह विश्वास है की यह विज्ञान और मानवता के लाभ के लिए है। मुझे इस शोधकार्य में होने वाले जोखिम के बारे में समझा दिया गया है और मेरी समझ में यह जोखिम बहुत मामुली है।

मैं इस शोधकार्य में भाग लेने के सम्बन्ध में किसी भी समय अपनी अस्वीकृति व्यक्त कर सकता/सकती हूँ और मेरे अस्वीकृति व्यक्त करने का मेरे इलाज पर कोई प्रभाव नहीं पड़ेगा।

_____ _____

गवाह के हस्ताक्षर रोगी के हस्ताक्षर/अंगूठे का निशान

नाम: नाम:

_____

अन्वेषक केहस्ताक्षर

_____

विद्यार्थी के हस्ताक्षर

ਮਰੀਜ਼ ਰਜ਼ਾਮੰਦੀ ਫਾਰਮ

ਮਿਤੀ_____

ਮੈ_____ ਪਤਨੀ/ਪੁੱਤਰ/ਪੁੱਤਰੀ_____ ਵਾਸੀ _____ **“EFFECT OF DELAYED LEUKOREDUCTION ON RBC AFTER PROLONG STORAGE BY USE OF INLINE FILTER”** ਤੇ ਚਲ ਰਹੀ ਖੋਜ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਰਜ਼ਾਮੰਦ ਹਾਂ। ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਮੈ ਆਪਣੀ ਮਰਜ਼ੀ ਨਾਲ ਤਿਆਰ ਹਾਂ। ਮੈ ਇਹ ਦਸਣਾ ਚਾਹੁੰਦਾ ਹਾਂ/ ਚਾਹੁੰਦੀ ਹਾਂ ਕੇ ਇਹ ਰਜ਼ਾਮੰਦੀ ਦੇਣ ਵਾਸਤੇ ਮੈਨੂ ਕਿਸੇ ਵੀ ਤਰਾਂ ਦਾ ਲੋਭ ਲਾਲਚ ਨਹੀ ਦਿੱਤਾ ਗਿਆ ਅਤੇ ਨਾਹੀ ਕੋਈ ਦਬਾ ਪਾਇਆ ਗਿਆ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਦਾਮਨੇਰਥ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਭਰੋਸਾ ਦਵਾਇਆ ਗਿਆ ਹੈ ਕੇ ਇਹ ਖੋਜ ਵਿਗਿਆਨ ਅਤੇ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਵਿਚ ਹੋਣ ਵਾਲੇ ਖਤਰੇ ਬਾਰੇ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਯਕੀਨ ਹੈ ਕੇ ਇਹ ਖਤਰਾ ਬਹੁਤ ਮਾਮੂਲੀ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਦਾ ਢੰਗ–ਤਰੀਕਾ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ।

ਮੈ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਬਾਰੇ ਆਪਣੀ ਰਜ਼ਾਮੰਦੀ ਕਿਸੇ ਵੀ ਸਮੇ ਵਾਪਸ ਲੈ ਸਕਦਾ ਹਾਂ/ ਸਕਦੀ ਹਾਂ ਅਤੇ ਮੇਰੀ ਰਜ਼ਾਮੰਦੀ ਵਾਪਸ ਲੈਣ ਦਾ ਮੇਰੇ ਇਲਾਜ ਤੇ ਕੋਈ ਅਸਰ ਨਹੀ ਹੋਵੇਗਾ।

_____ _____

ਗਵਾਹ ਦੇ ਹਸਤਾਖਰ ਮਰੀਜ਼ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____ ਮਿਤੀ_____

ਪਤਾ _____ ਪਤਾ _____

_____

ਨਿਰੀਖਕ ਦੇ ਹਸਤਾਖਰ

_____

ਸਿਖਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ